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(54) **POLYNUCLEOTIDE ENCODING A POLYPEPTIDE HAVING HEPARANASE ACTIVITY AND  
EXPRESSION OF SAME IN TRANSDUCED CELLS**

POLYNUKLEOTID KODIEREND FÜR EIN POLYPEPTID MIT HEPARANASE-AKTIVITÄT UND  
DESSEN EXPRESSION IN TRANSDUZIERTEN ZELLEN

POLYNUCLEOTIDE CODANT POUR UN POLYPEPTIDE PRESENTANT UNE ACTIVITE  
D'HEPARANASE ET SON EXPRESSION PAR DES CELLULES TRANSDUCTEES

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(56) References cited:  
**WO-A1-95/04158** **US-A- 5 362 641**

- JIN L ET AL: "MOLECULAR CLONING AND  
EXPRESSION OF HUMAN HEPARANASE CDNA"  
PROCEEDINGS AMERICAN ASSOCIATION FOR  
CANCER RESEARCH ANNUAL MEETING 1992,  
vol. 33, 1992, page 57 XP002139971 ISSN:  
0197-016X
- FREEMAN CRAIG ET AL: "Human platelet  
heparanase: Purification, characterization and  
catalytic activity." BIOCHEMICAL JOURNAL  
MARCH 15, 1998, vol. 330, no. 3, 15 March 1998  
(1998-03-15), pages 1341-1350, XP002139978  
ISSN: 0264-6021
- DATABASE GENBANK ON STN, US NATIONAL  
LIBRARY OF MEDICINE, (Bethesda, MD, USA),  
No. N32056, HILLIER et al., "The WashU-Merck  
EST Project", 10 January 1996, XP002915282

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- DATABASE GENBANK ON STN, US NATIONAL LIBRARY OF MEDICINE, (Bethesda, MD, USA), No. N30845, HILLIER et al., "The WashU- Merck EST Project", 05 January 1996, XP002915283
- DATABASE GENBANK ON STN, US NATIONAL LIBRARY OF MEDICINE, (Bethesda, MD, USA), No. N30824, HILLIER et al., "The WashU-Merck EST Project", 05 January 1996, XP002915284

- DATABASE GENBANK ON STN, US NATIONAL LIBRARY OF MEDICINE, (Bethesda, MD, USA), No. AA304653, ADAMS et al., "Initial Assessment of Human Gene Diversity and Expression Patterns Based Upon 83 Million Nucleotides of cDNA Sequence", 18 April 1997, XP002915285

## Description

FIELD AND BACKGROUND OF THE INVENTION

5 [0001] The present invention relates to a polynucleotide, referred to hereinbelow as *hpa*, encoding a polypeptide having heparanase activity, vectors including same and transduced cells expressing heparanase. The invention further relates to a recombinant protein having heparanase activity.

10 [0002] **Heparan sulfate proteoglycans:** Heparan sulfate proteoglycans (HSPG) are ubiquitous macromolecules associated with the cell surface and extra cellular matrix (ECM) of a wide range of cells of vertebrate and invertebrate tissues (1-4). The basic HSPG structure includes a protein core to which several linear heparan sulfate chains are covalently attached. These polysaccharide chains are typically composed of repeating hexuronic and D-glucosamine disaccharide units that are substituted to a varying extent with N- and O-linked sulfate moieties and N-linked acetyl groups (1-4). Studies on the involvement of ECM molecules in cell attachment, growth and differentiation revealed a central role of HSPG in embryonic morphogenesis, angiogenesis, neurite outgrowth and tissue repair (1-5). HSPG are prominent components of blood vessels (3). In large blood vessels they are concentrated mostly in the intima and inner media, whereas in capillaries they are found mainly in the subendothelial basement membrane where they support proliferating and migrating endothelial cells and stabilize the structure of the capillary wall. The ability of HSPG to interact with ECM macromolecules such as collagen, laminin and fibronectin, and with different attachment sites on plasma membranes suggests a key role for this proteoglycan in the self-assembly and insolubility of ECM components, as well as in cell adhesion and locomotion. Cleavage of the heparan sulfate (HS) chains may therefore result in degradation of the subendothelial ECM and hence may play a decisive role in extravasation of blood-borne cells. HS catabolism is observed in inflammation, wound repair, diabetes, and cancer metastasis, suggesting that enzymes which degrade HS play important roles in pathologic processes. Heparanase activity has been described in activated immune system cells and highly metastatic cancer cells (6-8), but research has been handicapped by the lack of biologic tools to explore potential causative roles of heparanase in disease conditions.

25 [0003] **Involvement of Heparanase in Tumor Cell Invasion and Metastasis:** Circulating tumor cells arrested in the capillary beds of different organs must invade the endothelial cell lining and degrade its underlying basement membrane (BM) in order to invade into the extravascular tissue(s) where they establish metastasis (9, 10). Metastatic tumor cells often attach at or near the intercellular junctions between adjacent endothelial cells. Such attachment of the metastatic cells is followed by rupture of the junctions, retraction of the endothelial cell borders and migration through the breach in the endothelium toward the exposed underlying BM (9). Once located between endothelial cells and the BM, the invading cells must degrade the subendothelial glycoproteins and proteoglycans of the BM in order to migrate out of the vascular compartment. Several cellular enzymes (e.g., collagenase IV, plasminogen activator, cathepsin B, elastase, etc.) are thought to be involved in degradation of BM (10). Among these enzymes is an endo- $\beta$ -D-glucuronidase (heparanase) that cleaves HS at specific intrachain sites (6, 8, 11). Expression of a HS degrading heparanase was found to correlate with the metastatic potential of mouse lymphoma (11), fibrosarcoma and melanoma (8) cells. Moreover, elevated levels of heparanase were detected in sera from metastatic tumor bearing animals and melanoma patients (8) and in tumor biopsies of cancer patients (12).

30 [0004] The control of cell proliferation and tumor progression by the local microenvironment, focusing on the interaction of cells with the extracellular matrix (ECM) produced by cultured corneal and vascular endothelial cells, was investigated previously by the present inventors. This cultured ECM closely resembles the subendothelium *in vivo* in its morphological appearance and molecular composition. It contains collagens (mostly type III and IV, with smaller amounts of types I and V), proteoglycans (mostly heparan sulfate- and dermatan sulfate- proteoglycans, with smaller amounts of chondroitin sulfate proteoglycans), laminin, fibronectin, entactin and elastin (13, 14). The ability of cells to degrade HS in the cultured ECM was studied by allowing cells to interact with a metabolically sulfate labeled ECM, followed by gel filtration (Sephacrose 6B) analysis of degradation products released into the culture medium (11). While intact HSPG are eluted next to the void volume of the column ( $K_{av} < 0.2$ ,  $M_r \sim 0.5 \times 10^6$ ), labeled degradation fragments of HS side chains are eluted more toward the  $V_t$  of the column ( $0.5 < k_{av} < 0.8$ ,  $M_r = 5-7 \times 10^3$ ) (11).

40 [0005] The heparanase inhibitory effect of various non-anticoagulant species of heparin that might be of potential use in preventing extravasation of blood-borne cells was also investigated by the present inventors. Inhibition of heparanase was best achieved by heparin species containing 16 sugar units or more and having sulfate groups at both the N and O positions. While O-desulfation abolished the heparanase inhibiting effect of heparin, O-sulfated, N-acetylated heparin retained a high inhibitory activity, provided that the N-substituted molecules had a molecular size of about 4,000 daltons or more (7). Treatment of experimental animals with heparanase inhibitors (e.g., non-anticoagulant species of heparin) markedly reduced (>90%) the incidence of lung metastases induced by B16 melanoma, Lewis lung carcinoma and mammary adenocarcinoma cells (7, 8, 16). Heparin fractions with high and low affinity to anti-thrombin III exhibited a comparable high anti-metastatic activity, indicating that the heparanase inhibiting activity of heparin, rather than its anticoagulant activity, plays a role in the anti-metastatic properties of the polysaccharide (7).

[0006] **Heparanase activity in the urine of cancer patients:** In an attempt to further elucidate the involvement of heparanase in tumor progression and its relevance to human cancer, urine samples for heparanase activity were screened (16a). Heparanase activity was detected in the urine of some, but not all, cancer patients. High levels of heparanase activity were determined in the urine of patients with an aggressive metastatic disease and there was no detectable activity in the urine of healthy donors.

[0007] Heparanase activity was also found in the urine of 20% of normal and microalbuminuric insulin dependent diabetes mellitus (IDDM) patients, most likely due to diabetic nephropathy, the most important single disorder leading to renal failure in adults.

[0008] **Possible involvement of heparanase in tumor angiogenesis:** Fibroblast growth factors are a family of structurally related polypeptides characterized by high affinity to heparin (17). They are highly mitogenic for vascular endothelial cells and are among the most potent inducers of neovascularization (17, 18). Basic fibroblast growth factor (bFGF) has been extracted from the subendothelial ECM produced *in vitro* (19) and from basement membranes of the cornea (20), suggesting that ECM may serve as a reservoir for bFGF. Immunohistochemical staining revealed the localization of bFGF in basement membranes of diverse tissues and blood vessels (21). Despite the ubiquitous presence of bFGF in normal tissues, endothelial cell proliferation in these tissues is usually very low, suggesting that bFGF is somehow sequestered from its site of action. Studies on the interaction of bFGF with ECM revealed that bFGF binds to HSPG in the ECM and can be released in an active form by HS degrading enzymes (15, 20, 22). It was demonstrated that heparanase activity expressed by platelets, mast cells, neutrophils, and lymphoma cells is involved in release of active bFGF from ECM and basement membranes (23), suggesting that heparanase activity may not only function in cell migration and invasion, but may also elicit an indirect neovascular response. These results suggest that the ECM HSPG provides a natural storage depot for bFGF and possibly other heparin-binding growth promoting factors (24, 25). Displacement of bFGF from its storage within basement membranes and ECM may therefore provide a novel mechanism for induction of neovascularization in normal and pathological situations.

[0009] Recent studies indicate that heparin and HS are involved in binding of bFGF to high affinity cell surface receptors and in bFGF cell signaling (26, 27). Moreover, the size of HS required for optimal effect was similar to that of HS fragments released by heparanase (28). Similar results were obtained with vascular endothelial cells growth factor (VEGF) (29), suggesting the operation of a dual receptor mechanism involving HS in cell interaction with heparin-binding growth factors. It is therefore proposed that restriction of endothelial cell growth factors in ECM prevents their systemic action on the vascular endothelium, thus maintaining a very low rate of endothelial cells turnover and vessel growth. On the other hand, release of bFGF from storage in ECM as a complex with HS fragment, may elicit localized endothelial cell proliferation and neovascularization in processes such as wound healing, inflammation and tumor development (24, 25).

[0010] **Expression of heparanase by cells of the immune system:** Heparanase activity correlates with the ability of activated cells of the immune system to leave the circulation and elicit both inflammatory and autoimmune responses. Interaction of platelets, granulocytes, T and B lymphocytes, macrophages and mast cells with the subendothelial ECM is associated with degradation of HS by a specific heparanase activity (6). The enzyme is released from intracellular compartments (e.g., lysosomes, specific granules, etc.) in response to various activation signals (e.g., thrombin, calcium ionophore, immune complexes, antigens, mitogens, etc.), suggesting its regulated involvement in inflammation and cellular immunity.

**Some of the observations regarding the heparanase enzyme were reviewed in reference No. 6 and are listed hereinbelow:**

[0011] First, a proteolytic activity (plasminogen activator) and heparanase participate synergistically in sequential degradation of the ECM HSPG by inflammatory leukocytes and malignant cells.

[0012] Second, a large proportion of the platelet heparanase exists in a latent form, probably as a complex with chondroitin sulfate. The latent enzyme is activated by tumor cell-derived factor(s) and may then facilitate cell invasion through the vascular endothelium in the process of tumor metastasis.

[0013] Third, release of the platelet heparanase from  $\alpha$ -granules is induced by a strong stimulant (i.e., thrombin), but not in response to platelet activation on ECM.

[0014] Fourth, the neutrophil heparanase is preferentially and readily released in response to a threshold activation and upon incubation of the cells on ECM.

[0015] Fifth, contact of neutrophils with ECM inhibited release of noxious enzymes (proteases, lysozyme) and oxygen radicals, but not of enzymes (heparanase, gelatinase) which may enable diapedesis. This protective role of the subendothelial ECM was observed when the cells were stimulated with soluble factors but not with phagocytosable stimulants.

[0016] Sixth, intracellular heparanase is secreted within minutes after exposure of T cell lines to specific antigens.

[0017] Seventh, mitogens (Con A, LPS) induce synthesis and secretion of heparanase by normal T and B lymphocytes maintained *in vitro*. T lymphocyte heparanase is also induced by immunization with antigen *in vivo*.

[0018] Eighth, heparanase activity is expressed by pre-B lymphomas and B-lymphomas, but not by plasmacytomas

and resting normal B lymphocytes.

[0019] Ninth, heparanase activity is expressed by activated macrophages during incubation with ECM, but there was little or no release of the enzyme into the incubation medium. Similar results were obtained with human myeloid leukemia cells induced to differentiate to mature macrophages.

5 [0020] Tenth, T-cell mediated delayed type hypersensitivity and experimental autoimmunity are suppressed by low doses of heparanase inhibiting non-anticoagulant species of heparin (30).

[0021] Eleventh, heparanase activity expressed by platelets, neutrophils and metastatic tumor cells releases active bFGF from ECM and basement membranes. Release of bFGF from storage in ECM may elicit a localized neovascular response in processes such as wound healing, inflammation and tumor development.

10 [0022] Twelfth, among the breakdown products of the ECM generated by heparanase is a tri-sulfated disaccharide that can inhibit T-cell mediated inflammation *in vivo* (31). This inhibition was associated with an inhibitory effect of the disaccharide on the production of biologically active TNF $\alpha$  by activated T cells *in vitro* (31).

[0023] **Other potential therapeutic applications:** Apart from its involvement in tumor cell metastasis, inflammation and autoimmunity, mammalian heparanase may be applied to modulate: bioavailability of heparin-binding growth factors (15); cellular responses to heparin-binding growth factors (e.g., bFGF, VEGF) and cytokines (IL-8) (31 a, 29); cell interaction with plasma lipoproteins (32); cellular susceptibility to certain viral and some bacterial and protozoa infections (33, 33a, 33b); and disintegration of amyloid plaques (34). Heparanase may thus prove useful for conditions such as wound healing, angiogenesis, restenosis, atherosclerosis, inflammation, neurodegenerative diseases and viral infections. Mammalian heparanase can be used to neutralize plasma heparin; as a potential replacement of protamine.

20 Anti-heparanase antibodies may be applied for immunodetection and diagnosis of micrometastases, autoimmune lesions and renal failure in biopsy specimens, plasma samples, and body fluids. Common use in basic research is expected.

[0024] The identification of the *hpa* gene encoding for heparanase enzyme will enable the production of a recombinant enzyme in heterologous expression systems. Availability of the recombinant protein will pave the way for solving the protein structure function relationship and will provide a tool for developing new inhibitors.

25 [0025] **Viral Infection:** The presence of heparan sulfate on cell surfaces have been shown to be the principal requirement for the binding of Herpes Simplex (33) and Dengue (33a) viruses to cells and for subsequent infection of the cells. Removal of the cell surface heparan sulfate by heparanase may therefore abolish virus infection. In fact, treatment of cells with bacterial heparitinase (degrading heparan sulfate) or heparinase (degrading heparan) reduced the binding of two related animal herpes viruses to cells and rendered the cells at least partially resistant to virus infection (33). There are some indications that the cell surface heparan sulfate is also involved in HIV infection (33b).

30 [0026] **Neurodegenerative diseases:** Heparan sulfate proteoglycans were identified in the prion protein amyloid plaques of Genstmann-Straussler Syndrome, Creutzfeldt-Jakob disease and Scrape (34). Heparanase may disintegrate these amyloid plaques which are also thought to play a role in the pathogenesis of Alzheimer's disease.

35 [0027] **Restenosis and Atherosclerosis:** Proliferation of arterial smooth muscle cells (SMCs) in response to endothelial injury and accumulation of cholesterol rich lipoproteins are basic events in the pathogenesis of atherosclerosis and restenosis (35). Apart from its involvement in SMC proliferation (i.e., low affinity receptors for heparin-binding growth factors), HS is also involved in lipoprotein binding, retention and uptake (36). It was demonstrated that HSPG and lipoprotein lipase participate in a novel catabolic pathway that may allow substantial cellular and interstitial accumulation of cholesterol rich lipoproteins (32). The latter pathway is expected to be highly atherogenic by promoting accumulation of apoB and apoE rich lipoproteins (i.e. LDL, VLDL, chylomicrons), independent of feed back inhibition by the cellular sterol content. Removal of SMC HS by heparanase is therefore expected to inhibit both SMC proliferation and lipid accumulation and thus may halt the progression of restenosis and atherosclerosis.

40 [0028] There is thus a widely recognized need for, and it would be highly advantageous to have a polynucleotide encoding a polypeptide having heparanase activity, vectors including same, transduced cells expressing heparanase and a recombinant protein having heparanase activity.

#### SUMMARY OF THE INVENTION

50 [0029] According to the present invention there is provided a polynucleotide, referred to hereinbelow as *hpa*, *hpa* cDNA or *hpa* gene, encoding a polypeptide having heparanase activity, vectors including same, transduced cells expressing heparanase and a recombinant protein having heparanase activity.

[0030] Cloning of the human *hpa* gene which encodes heparanase, and expression of recombinant heparanase by transfected host cells is reported.

55 [0031] A purified preparation of heparanase isolated from human hepatoma cells was subjected to tryptic digestion and microsequencing. The YGPDVGQPR (SEQ ID NO:8) sequence revealed was used to screen EST databases for homology to the corresponding back translated DNA sequence. Two closely related EST sequences were identified and were thereafter found to be identical. Both clones contained an insert of 1020 bp which included an open reading frame of 973 bp followed by a 27 bp of 3' untranslated region and a Poly. A tail. Translation start site was not identified.



[0032] Cloning of the missing 5' end of *hpa* was performed by PCR amplification of DNA from placenta Marathon RACE (RIM) cDNA composite using primers selected according to the EST clones sequence and the linkers of the composite. A 900 bp PCR fragment, partially overlapping with the identified 3' encoding EST clones was obtained. The joined cDNA fragment (*hpa*), 1721 bp long (SEQ ID NO:9), contained an open reading frame which encodes a polypeptide of 543 amino acids (SEQ ID NO:10) with a calculated molecular weight of 61,192 daltons.

[0033] Cloning an extended 5' sequence was enabled from the human SK-hepl cell line by PCR amplification using the Marathon RACE (RIM). The 5' extended sequence of the SK-hep 1 *hpa* cDNA was assembled with the sequence of the *hpa* cDNA isolated from human placenta (SEQ ID NO:9). The assembled sequence contained an open reading frame, SEQ ID NOs: 13 and 15, which encodes, as shown in SEQ ID NOs: 14 and 15, a polypeptide of 592 amino acids with, a calculated molecular weight of 66,407 daltons.

[0034] The ability of the *hpa* gene product to catalyze degradation of heparan sulfate in an *in vitro* assay was examined by expressing the entire open reading frame of *hpa* in insect cells, using the Baculovirus expression system. Extracts and conditioned media of cells infected with virus containing the *hpa* gene, demonstrated a high level of heparan sulfate degradation activity both towards soluble ECM-derived HSPG and intact ECM. This degradation activity was inhibited by heparin, which is another substrate of heparanase. Cells infected with a similar construct containing no *hpa* gene had no such activity, nor did non-infected cells. The ability of heparanase expressed from the extended 5' clone towards heparin was demonstrated in a mammalian expression system.

[0035] The expression pattern of *hpa* RNA in various tissues and cell lines was investigated using RT-PCR. It was found to be expressed only in tissues and cells previously known to have heparanase activity.

[0036] A panel of monochromosomal human/CHO and human/mouse somatic cell hybrids was used to localize the human heparanase gene to human chromosome 4. The newly isolated heparanase sequence can be used to identify a chromosome region harboring a human heparanase gene in a chromosome spread.

[0037] According to further features in preferred embodiments of the invention described below, there is provided a polynucleotide fragment which includes a polynucleotide sequence encoding a polypeptide having heparanase catalytic activity.

[0038] According to still further features in the described preferred embodiments the polynucleotide fragment includes nucleotides 63-1691 of SEQ ID NO:9 or nucleotides 139-1869 of SEQ ID NO:13, which encode the entire human heparanase enzyme.

[0039] According to still further features in the described preferred embodiments there is provided a polynucleotide fragment which includes a polynucleotide sequence capable of hybridizing with *hpa* cDNA, especially with nucleotides 1-721 of SEQ ID NO:9.

[0040] According to still further features in the described preferred embodiments the polynucleotide sequence which encodes the polypeptide having heparanase activity shares at least 60 % homology, preferably at least 70 % homology, more preferably at least 80 % homology, most preferably at least 90 % homology with SEQ ID NOs:9 or 13.

[0041] According to still further features in the described preferred embodiments the polynucleotide fragment according to the present invention includes a portion (fragment) of SEQ ID NOs:9, or 13. For example, such fragments could include nucleotides 63-721 of SEQ ID NO:9 and/or a segment of SEQ ID NO:9 which encodes a polypeptide having the heparanase catalytic activity.

[0042] According to still further features in the described preferred embodiments the polypeptide encoded by the polynucleotide fragment includes an amino acid sequence as set forth in SEQ ID NOs:10 or 14 or a functional part thereof.

[0043] According to still further features in the described preferred embodiments the polynucleotide sequence encodes a polypeptide having heparanase activity, which shares at least 60 % homology, preferably at least 70 % homology, more preferably at least 80 % homology, most preferably at least 90 % homology with SEQ ID NOs:10 or 14.

[0044] According to still further features in the described preferred embodiments the polynucleotide fragment encodes a polypeptide having heparanase activity, which may therefore be allelic, species and/or induced variant of the amino acid sequence set forth in SEQ ID NOs:10 or 14. It is understood that any such variant may also be considered a homolog.

[0045] According to still further features in the described preferred embodiments there is provided a single stranded polynucleotide fragment which includes a polynucleotide sequence complementary to at least a portion of a polynucleotide strand encoding a polypeptide having heparanase catalytic activity as described above.

[0046] According to still further features in the described preferred embodiments there is provided a vector including a polynucleotide sequence encoding a polypeptide having heparanase catalytic activity.

[0047] The vector may be of any suitable type including but not limited to a phage, virus, plasmid, phagemid, cosmid, bacmid or even an artificial chromosome. The polynucleotide sequence encoding a polypeptide having heparanase catalytic activity may include any of the above described polynucleotide fragments.

[0048] According to still further features in the described preferred embodiments there is provided a host cell which includes an exogenous polynucleotide fragment including a polynucleotide sequence encoding a polypeptide having heparanase catalytic activity.

[0049] The exogenous polynucleotide fragment may be any of the above described fragments. The host cell may be

of any type such as prokaryotic cell, eukaryotic cell, a cell line, or a cell as a portion of a multicellular organism (e.g., cells of a transgenic organism).

[0050] According to still further features in the described preferred embodiments there is provided a recombinant protein including a polypeptide having heparanase catalytic activity.

[0051] According to still further features in the described preferred embodiments there is provided a pharmaceutical composition comprising as an active ingredient a recombinant protein having heparanase catalytic activity.

[0052] According to still further features in the described preferred embodiments there is provided a medical equipment comprising a medical device containing, as an active ingredient a recombinant protein having heparanase catalytic activity.

[0053] According to still further features in the described preferred embodiments there is provided a heparanase overexpression system comprising a cell overexpressing heparanase catalytic activity.

[0054] According to still further features in the described preferred embodiments there is provided a method of identifying a chromosome region harboring a human heparanase gene in a chromosome spread comprising the steps of (a) hybridizing the chromosome spread with a tagged polynucleotide probe encoding heparanase; (b) washing the chromosome spread, thereby removing excess of non-hybridized probe; and (c) searching for signals associated with said hybridized tagged polynucleotide probe, wherein detected signals being indicative of a chromosome region harboring a human heparanase gene.

[0055] The present invention can be used to develop new drugs to inhibit tumor cell metastasis, inflammation and autoimmunity. The identification of the *hpa* gene encoding for heparanase enzyme enables the production of a recombinant enzyme in heterologous expression systems.

#### BRIEF DESCRIPTION OF THE DRAWINGS

[0056] The invention herein described, by way of example only, with reference to the accompanying drawings, wherein:

FIG. 1 presents nucleotide sequence and deduced amino acid sequence of *hpa* cDNA. A single nucleotide difference at position 799 (A to T) between the EST (Expressed Sequence Tag) and the PCR amplified cDNA (reverse transcribed RNA) and the resulting amino acid substitution (Tyr to Phe) are indicated above and below the substituted unit, respectively. Cysteine residues and the poly adenylation consensus sequence are underlined. The asterisk denotes the stop codon TGA.

FIG. 2 demonstrates degradation of soluble sulfate labeled HSPG substrate by lysates of High Five (RIM) cells infected with pFhpa2 virus. Lysates of High Five (RIM) cells that were infected with pFhpa2 virus (•) or control pF2 virus (□) were incubated (18 h, 37 °C) with sulfate labeled ECM-derived soluble HSPG (peak I). The incubation medium was then subjected to gel filtration on Sepharose 6B. Low molecular weight HS degradation fragments (peak II) were produced only during incubation with the pFhpa2 infected cells, but there was no degradation of the HSPG substrate (♦) by lysates of pF2 infected cells.

FIGs. 3a-b demonstrate degradation of soluble sulfate labeled HSPG substrate by the culture medium of pFhpa2 and pFhpa4 infected cells. Culture media of High Five (RIM) cells infected with pFhpa2 (3a) or pFhpa4 (3b) viruses (•), or with control viruses (□) were incubated (18 h, 37 °C) with sulfate labeled ECM-derived soluble HSPG (peak I, ♦). The incubation media were then subjected to gel filtration on Sepharose 6B. Low molecular weight HS degradation fragments (peak II) were produced only during incubation with the *hpa* gene containing viruses. There was no degradation of the HSPG substrate by the culture medium of cells infected with control viruses.

FIG. 4 presents size fractionation of heparanase activity expressed by pFhpa2 infected cells. Culture medium of pFhpa2 infected High Five (RIM) cells was applied onto a 50 kDa cut-off membrane. Heparanase activity (conversion of the peak I substrate, (♦) into peak II HS degradation fragments) was found in the high (> 50 kDa) (•), but not low (< 50 kDa) (o) molecular weight compartment.

FIGs. 5a-b demonstrate the effect of heparin on heparanase activity expressed by pFhpa2 and pFhpa4 infected High Five (RIM) cells. Culture media of pFhpa2 (5a) and pFhpa4 (5b) infected High Five (RIM) cells were incubated (18 h, 37 °C) with sulfate labeled ECM-derived soluble HSPG (peak I, ♦) in the absence (•) or presence (Δ) of 10 μg/ml heparin. Production of low molecular weight HS degradation fragments was completely abolished in the presence of heparin, a potent inhibitor of heparanase activity (6, 7).

FIGs. 6a-b demonstrate degradation of sulfate labeled intact ECM by virus infected High Five (RIM) and Sf21 Cells. High Five (RIM) (6a) and Sf21 (6b) cells were plated on sulfate labeled ECM and infected (48 h, 28 °C) with pFhpa4 (•) or control pF1 (□) viruses. Control non-infected Sf21 cells (R) were plated on the labeled ECM as well. The pH of the cultured medium was adjusted to 6.0 - 6.2 followed by 24 h incubation at 37 °C. Sulfate labeled material released into the incubation medium was analyzed by gel filtration on Sepharose 6B. HS degradation fragments were produced only by cells infected with the *hpa* containing virus.

FIG. 7a-b demonstrate degradation of sulfate labeled intact ECM by virus infected cells. High Five (RIM) (7a) and

Sf21 (7b) cells were plated on sulfate labeled ECM and infected (48 h, 28 °C) with pF*hpa*4 (•) or control pF1 (□) viruses. Control non-infected Sf21 cells (R) were plate on labeled ECM as well. The pH of the cultured medium was adjusted to 6.0 - 6.2, followed by 48 h incubation at 28 °C. Sulfate labeled degradation fragments released into the incubation medium was analyzed by gel filtration on Sepharose 6B. HS degradation fragments were produced only by cells infected with the *hpa* containing virus.

FIGs. 8a-b demonstrate degradation of sulfate labeled intact ECM by the culture medium of pF*hpa*4 infected cells. Culture media of High Five (RIM) (8a) and Sf21 (8b) cells that were infected with pF*hpa*4 (•) or control pF1 (□) viruses were incubated (48 h, 37 °C, pH 6.0) with intact sulfate labeled ECM. The ECM was also incubated with the culture medium of control non-infected Sf21 cells (R). Sulfate labeled material released into the reaction mixture was subjected to gel filtration analysis. Heparanase activity was detected only in the culture medium of pF*hpa*4 infected cells.

FIGs. 9a-b demonstrate the effect of heparin on heparanase activity in the culture medium of pF*hpa*4 infected cells. Sulfate labeled ECM was incubated (24 h, 37 °C, pH 6.0) with culture medium of pF*hpa*4 infected High Five (RIM) (9a) and Sf2 (9b) cells in the absence (•) or presence (V) of 10 µg/ml heparin. Sulfate labeled material released into the incubation medium was subjected to gel filtration on Sepharose 6B. Heparanase activity (production of peak II HS degradation fragments) was completely inhibited in the presence of heparin.

FIGs. 10a-b demonstrate purification of recombinant heparanase on heparin-Sepharose. Culture medium of Sf21 cells infected with pF*hpa*4 virus was subjected to heparin-Sepharose chromatography. Elution of fractions was performed with 0.3 - 2 M NaCl gradient (♦). Heparanase activity in the eluted fractions is demonstrated in Figure 10a (•). Fractions 15-28 were subjected to 15% SDS-polyacrylamide gel electrophoresis followed by silver nitrate staining. A correlation is demonstrated between a major protein band (MW ~ 63,000) in fractions 19-24 and heparanase activity.

FIGs. 11a-b demonstrate purification of recombinant heparanase on a Superdex 75 gel filtration column. Active fractions eluted from heparin-Sepharose (Figure 10a) were pooled, concentrated and applied onto Superdex 75 FPLC column. Fractions were collected and aliquots of each fraction were tested for heparanase activity (C, Figure 11a) and analyzed by SDS-polyacrylamide gel electrophoresis followed by silver nitrate staining (Figure 11b). A correlation is seen between the appearance of a major protein band (MW ~ 63,000) in fractions 4 - 7 and heparanase activity.

FIGs. 12a-e demonstrate expression of the *hpa* gene by RT-PCR with total RNA from human embryonal tissues (12a), human extra-embryonal tissues (12b) and cell lines from different origins (12c-e). RT-PCR products using *hpa* specific primers (I), primers for GAPDH housekeeping gene (II), and control reactions without reverse transcriptase demonstrating absence of genomic DNA or other contamination in RNA samples (III). M- DNA molecular weight marker VI (Boehringer Mannheim). For 12a: lane 1 - neutrophil cells (adult), lane 2 - muscle, lane 3 - thymus, lane 4 - heart, lane 5 - adrenal. For 12b: lane 1 - kidney, lane 2 - placenta (8 weeks), lane 3 - placenta (11 weeks), lanes 4-7 - mole (complete hydatidiform mole), lane 8 - cytotrophoblast cells (freshly isolated), lane 9 - cytotrophoblast cells (1.5 h *in vitro*), lane 10 - cytotrophoblast cells (6 h *in vitro*), lane 11 - cytotrophoblast cells (18 h *in vitro*), lane 12 - cytotrophoblast cells (48 h *in vitro*). For 12c: lane 1 - JAR bladder cell line, lane 2 - NCITT testicular tumor cell line, lane 3 - SW-480 human hepatoma cell line, lane 4 - HTR (cytotrophoblasts transformed by SV40), lane 5 - HPTLP-1 hepatocellular carcinoma cell line, lane 6 - EJ-28 bladder carcinoma cell line. For 12d: lane 1 - SK-hep-1 human hepatoma cell line, lane 2 - DAMI human megakaryocytic cell line, lane 3 - DAMI cell line + PMA, lane 4 - CHRF cell line + PMA, lane 5 - CHRF cell line. For 12e: lane 1 - ABAE bovine aortic endothelial cells, lane 2 - 1063 human ovarian cell line, lane 3 - human breast carcinoma MDA435 cell line, lane 4 - human breast carcinoma MDA231 cell line.

FIG. 13 presents a comparison between nucleotide sequences of the human *hpa* and a mouse EST cDNA fragment (SEQ ID NO:12) which is 80 % homologous to the 3' end (starting at nucleotide 1066 of SEQ ID NO:9) of the human *hpa*. The aligned termination codons are underlined.

FIG. 14 demonstrates the chromosomal localization of the *hpa* gene. PCR products of DNA derived from somatic cell hybrids and of genomic DNA of hamster, mouse and human of were separated on 0.7 % agarose gel following amplification with *hpa* specific primers. Lane 1 - Lambda DNA digested with *Bst*EII, lane 2 - no DNA control, lanes 3 - 29, PCR amplification products. Lanes 3-5 - human, mouse and hamster genomic DNA, respectively. Lanes 6-29, human monochromosomal somatic cell hybrids representing chromosomes 1-22 and X and Y, respectively. Lane 30 - Lambda DNA digested with *Bst*EII. An amplification product of approximately 2.8 Kb is observed only in lanes 5 and 9, representing human genomic DNA and DNA derived from cell hybrid carrying human chromosome 4, respectively. These results demonstrate that the *hpa* gene is localized in human chromosome 4.

#### DESCRIPTION OF THE PREFERRED EMBODIMENTS

[0057] The present invention is of a polynucleotide, referred to hereinbelow interchangeably as *hpa*, *hpa* cDNA or



*hpa* gene, encoding a polypeptide having heparanase activity, vectors including same, transduced cells expressing heparanase and a recombinant protein having heparanase activity.

**[0058]** Before explaining at least one embodiment of the invention in detail, it is to be understood that the invention is not limited in its application to the details of construction and the arrangement of the components set forth in the following description or illustrated in the drawings.

**[0059]** Also, it is to be understood that the phraseology and terminology employed herein is for the purpose of description and should not be regarded as limiting.

**[0060]** The present invention can be used to develop treatments for various diseases, to develop diagnostic assays for these diseases and to provide new tools for basic research especially in the fields of medicine and biology.

**[0061]** Specifically, the present invention can be used to develop new drugs to inhibit tumor cell metastasis, inflammation and autoimmunity. The identification of the *hpa* gene encoding for the heparanase enzyme enables the production of a recombinant enzyme in heterologous expression systems.

**[0062]** Furthermore, the present invention can be used to modulate bioavailability of heparin-binding growth factors, cellular responses to heparin-binding growth factors (e.g., bFGF, VEGF) and cytokines (IL-8), cell interaction with plasma lipoproteins, cellular susceptibility to viral, protozoa and some bacterial infections, and disintegration of neurodegenerative plaques. Recombinant heparanase is thus a potential treatment for wound healing, angiogenesis, restenosis, atherosclerosis, inflammation, neurodegenerative diseases (such as, for example, Genstmann-Straussler Syndrome, Creutzfeldt-Jakob disease, Scrapie and Alzheimer's disease) and certain viral and some bacterial and protozoa infections. Recombinant heparanase can be used to neutralize plasma heparin, as a potential replacement of protamine.

**[0063]** As used herein, the term "modulate" includes substantially inhibiting, slowing or reversing the progression of a disease, substantially ameliorating clinical symptoms of a disease or condition, or substantially preventing the appearance of clinical symptoms of a disease or condition. A "modulator" therefore includes an agent which may modulate a disease or condition. Modulation of viral, protozoa and bacterial infections includes any effect which substantially interrupts, prevents or reduces any viral, bacterial or protozoa activity and/or stage of the virus, bacterium or protozoon life cycle, or which reduces or prevents infection by the virus, bacterium or protozoon in a subject, such as a human or lower animal.

**[0064]** As used herein, the term "wound" includes any injury to any portion of the body of a subject including, but not limited to, acute conditions such as thermal burns, chemical burns, radiation burns, burns caused by excess exposure to ultraviolet radiation such as sunburn, damage to bodily tissues such as the perineum as a result of labor and childbirth, including injuries sustained during medical procedures such as episiotomies, trauma-induced injuries including cuts, those injuries sustained in automobile and other mechanical accidents, and those caused by bullets, knives and other weapons, and post-surgical injuries, as well as chronic conditions such as pressure sores, bedsores, conditions related to diabetes and poor circulation, and all types of acne, etc.

**[0065]** Anti-heparanase antibodies, raised against the recombinant enzyme, would be useful for immunodetection and diagnosis of micrometastases, autoimmune lesions and renal failure in biopsy specimens, plasma samples, and body fluids. Such antibodies may also serve as neutralizing agents for heparanase activity.

**[0066]** Cloning of the human *hpa* gene encoding heparanase and expressing recombinant heparanase by transfected cells is herein reported. This is the first mammalian heparanase gene to be cloned.

**[0067]** A purified preparation of heparanase isolated from human hepatoma cells was subjected to tryptic digestion and microsequencing.

**[0068]** The YGPDVGQPR (SEQ ID NO:8) sequence revealed was used to screen EST databases for homology to the corresponding back translated DNA sequences. Two closely related EST sequences were identified and were thereafter found to be identical.

**[0069]** Both clones contained an insert of 1020 bp which includes an open reading frame of 973 bp followed by a 3' untranslated region of 27 bp and a Poly A tail, whereas a translation start site was not identified.

**[0070]** Cloning of the missing 5' end was performed by PCR amplification of DNA from placenta marathon RACE (RIM) cDNA composite using primers selected according to the EST clones sequence and the linkers of the composite.

**[0071]** A 900 bp PCR fragment, partially overlapping with the identified 3' encoding EST clones was obtained. The joined cDNA fragment (*hpa*), 1721 bp long (SEQ ID NO:9), contained an open reading frame which encodes, as shown in Figure 1 and SEQ ID NO:11, a polypeptide of 543 amino acids (SEQ ID NO:10) with a calculated molecular weight of 61,192 daltons.

**[0072]** A single nucleotide difference at position 799 (A to T) between the EST clones and the PCR amplified cDNA was observed. This difference results in a single amino acid substitution (Tyr to Phe) (Figure 1). Furthermore, the published EST sequences contained an unidentified nucleotide, which following DNA sequencing of both the EST clones was resolved into two nucleotides (G and C at positions 1630 and 1631 in SEQ ID NO:9, respectively).

**[0073]** The ability of the *hpa* gene product to catalyze degradation of heparan sulfate in an *in vitro* assay was examined by expressing the entire open reading frame in insect cells, using the Baculovirus expression system.

**[0074]** Extracts and conditioned media of cells infected with virus containing the *hpa* gene, demonstrated a high level

of heparan sulfate degradation activity both towards soluble ECM-derived HSPG and intact ECM, which was inhibited by heparin, while cells infected with a similar construct containing no *hpa* gene had no such activity, nor did non-infected cells.

[0075] The expression pattern of *hpa* RNA in various tissues and cell lines was investigated using RT-PCR. It was found to be expressed only in tissues and cells previously known to have heparanase activity.

[0076] Cloning an extended 5' sequence was enabled from the human SK-hep1 cell line by PCR amplification using the Marathon RACE (RIM) The 5' extended sequence of the SK-hep1 *hpa* cDNA was assembled with the sequence of the *hpa* cDNA isolated from human placenta (SEQ ID NO:9). The assembled sequence contained an open reading frame, SEQ ID NOs: 13 and 15, which encodes, as shown in SEQ ID NOs:14 and 15, a polypeptide of 592 amino acids, with a calculated molecular weight of 66,407 daltons. This open reading frame was shown to direct the expression of catalitically active heparanase in a mammalian cell expression system. The expressed heparanase was detectable by anti heparanase antibodies in Western blot analysis.

[0077] A panel of monochromosomal human/CHO and human/mouse somatic cell hybrids was used to localize the human heparanase gene to human chromosome 4. The newly isolated heparanase sequence can therefore be used to identify a chromosome region harboring a human heparanase gene in a chromosome spread.

[0078] Thus, according to the present invention there is provided a polynucleotide fragment (either DNA or RNA, either single stranded or double stranded) which includes a polynucleotide sequence encoding a polypeptide having heparanase catalytic activity.

[0079] The term "heparanase catalytic activity" or its equivalent term "heparanase activity" both refer to a mammalian endoglycosidase hydrolyzing activity which is specific for heparan or heparan sulfate proteoglycan substrates, as opposed to the activity of bacterial enzymes (heparinase I, II and III) which degrade heparin or heparan sulfate by means of  $\beta$ -elimination (37).

[0080] In a preferred embodiment of the invention the polynucleotide fragment includes nucleotides 63-1691 of SEQ ID NO:9, or nucleotides 139-1869 of SEQ ID NO: 13, which encode the entire human heparanase enzyme.

[0081] However, the scope of the present invention is not limited to human heparanase since this is the first disclosure of an open reading frame (ORF) encoding any mammalian heparanase. Using the *hpa* cDNA, parts thereof or synthetic oligonucleotides designed according to its sequence will enable one ordinarily skilled in the art to identify genomic and/or cDNA clones including homologous sequences from other mammalian species.

[0082] The present invention is therefore further directed at a polynucleotide fragment which includes a polynucleotide sequence capable of hybridizing (base pairing under either stringent or permissive hybridization conditions, as for example described in Sambrook, J.; Fritsch, E.F., Maniatis, T. (1989) Molecular Cloning. A Laboratory Manual. Cold Spring Harbor Laboratory Press, New York.) with *hpa* cDNA, especially with nucleotides 1-721 of SEQ ID NO:9.

[0083] In fact, any polynucleotide sequence which encodes a polypeptide having heparanase activity and which shares at least 60 % homology, preferably at least 70 % homology, more preferably at least 80 % homology, most preferably at least 90 % homology with SEQ ID NOs:9 or 13 is within the scope of the present invention.

[0084] The polynucleotide fragment according to the present invention may include any part of SEQ ID NOs: 9 or 13. For example, it may include nucleotides 63-721 of SEQ ID NO:9, which is a novel sequence. However, it may include any segment of SEQ ID NOs:9 or 13 which encodes a polypeptide having the heparanase catalytic activity.

[0085] When the phrase "encodes a polypeptide having heparanase catalytic activity" is used herein and in the claims section below it refers to the ability of directing the synthesis of a polypeptide which, if so required for its activity, following post translational modifications, such as but not limited to, proteolysis (e.g., removal of a signal peptide and of a pro- or preprotein sequence), methionine modification, glycosylation, alkylation (e.g., methylation), acetylation, etc., is catalytically active in degradation of, for example, ECM and cell surface associated HS.

[0086] In a preferred embodiment of the invention the polypeptide encoded by the polynucleotide fragment includes an amino acid sequence as set forth in SEQ ID NOs:10 or 14 or a functional part thereof, i.e., a portion harboring heparanase catalytic activity.

[0087] However, any polynucleotide fragment which encodes a polypeptide having heparanase activity is within the scope of the present invention. Therefore, the polypeptide may be allelic, species and/or induced variant of the amino acid sequence set forth in SEQ ID NOs: 10 or 14 or functional part thereof.

[0088] In fact, any polynucleotide sequence which encodes a polypeptide having heparanase activity, which shares at least 60 % homology, preferably at least 70 % homology, more preferably at least 80 % homology, most preferably at least 90 % homology with SEQ ID NOs:10 or 14 is within the scope of the present invention.

[0089] The invention is also directed at providing a single stranded polynucleotide fragment which includes a polynucleotide sequence complementary to at least a portion of a polynucleotide strand encoding a polypeptide having heparanase catalytic activity as described above. The term "complementary" as used herein refers to the ability of base pairing.

[0090] The single stranded polynucleotide fragment may be DNA or RNA or even include nucleotide analogs (e.g., thioated nucleotides), it may be a synthetic oligonucleotide or manufactured by transduced host cells, it may be of any desired length which still provides specific base pairing (e.g., 8 or 10, preferably more, nucleotides long) and it may

include mismatches that do not hamper base pairing.

**[0091]** The invention is further directed at providing a vector which includes a polynucleotide sequence encoding a polypeptide having heparanase catalytic activity.

**[0092]** The vector may be of any type. It may be a phage which infects bacteria or a virus which infects eukaryotic cells. It may also be a plasmid, phagemid, cosmid, bacmid or an artificial chromosome. The polynucleotide sequence encoding a polypeptide having heparanase catalytic activity may include any of the above described polynucleotide fragments.

**[0093]** The invention is further directed at providing a host cell which includes an exogenous polynucleotide fragment encoding a polypeptide having heparanase catalytic activity.

**[0094]** The exogenous polynucleotide fragment may be any of the above described fragments. The host cell may be of any type. It may be a prokaryotic cell, an eukaryotic cell, a cell line, or a cell as a portion of an organism. The exogenous polynucleotide fragment may be permanently or transiently present in the cell. In other words, transduced cells obtained following stable or transient transfection, transformation or transduction are all within the scope of the present invention. The term "exogenous" as used herein refers to the fact that the polynucleotide fragment is externally introduced into the cell. Therein it may be present in a single of any number of copies, it may be integrated into one or more chromosomes at any location or be present as an extrachromosomal material.

**[0095]** The invention is further directed at providing a heparanase overexpression system which includes a cell overexpressing heparanase catalytic activity. The cell may be a host cell transiently or stably transfected or transformed with any suitable vector which includes a polynucleotide sequence encoding a polypeptide having heparanase activity and a suitable promoter and enhancer sequences to direct overexpression of heparanase. However, the overexpressing cell may also be a product of an insertion (e.g., via homologous recombination) of a promoter and/or enhancer sequence upstream to the endogenous heparanase gene of the expressing cell, which will direct overexpression from the endogenous gene. The term "overexpression" as used herein in the specification and claims below refers to a level of expression which is higher than a basal level of expression typically characterizing a given cell under otherwise identical conditions.

**[0096]** The invention is further directed at providing a recombinant protein including a polypeptide having heparanase catalytic activity.

**[0097]** The recombinant protein may be purified by any conventional protein purification procedure close to homogeneity and/or be mixed with additives. The recombinant protein may be manufactured using any of the cells described above. The recombinant protein may be in any form. It may be in a crystallized form, a dehydrated powder form or in solution. The recombinant protein may be useful in obtaining pure heparanase, which in turn may be useful in eliciting anti-heparanase antibodies, either poly or monoclonal antibodies, and as a screening active ingredient in an anti-heparanase inhibitors or drugs screening assay or system.

**[0098]** The invention is further directed at providing a pharmaceutical composition which include as an active ingredient a recombinant protein having heparanase catalytic activity.

**[0099]** Formulations for topical administration may include, but are not limited to, lotions, ointments, gels, creams, suppositories, drops, liquids, sprays and powders. Conventional pharmaceutical carriers, aqueous, powder or oily bases, thickeners and the like may be necessary or desirable. Coated condoms, stents, active pads, and other medical devices may also be useful. In fact the scope of the present invention includes any medical equipment such as a medical device containing, as an active ingredient, a recombinant protein having heparanase catalytic activity.

**[0100]** Compositions for oral administration include powders or granules, suspensions or solutions in water or non-aqueous media, sachets, capsules or tablets. Thickeners, diluents, flavorings, dispersing aids, emulsifiers or binders may be desirable.

**[0101]** Formulations for parenteral administration may include, but are not limited to; sterile aqueous solutions which may also contain buffers, diluents and other suitable additives.

**[0102]** Dosing is dependent on severity and responsiveness of the condition to be treated, but will normally be one or more doses per day, with course of treatment lasting from several days to several months or until a cure is effected or a diminution of disease state is achieved. Persons ordinarily skilled in the art can easily determine optimum dosages, dosing methodologies and repetition rates.

**[0103]** Further according to the present invention there is provided a method of identifying a chromosome region harboring a human heparanase gene in a chromosome spread. the method is executed implementing the following method steps, in which in a first step the chromosome spread (either interphase or metaphase spread) is hybridized with a tagged polynucleotide probe encoding heparanase. The tag is preferably a fluorescent tag. In a second step according to the method the chromosome spread is washed, thereby excess of non-hybridized probe is removed. Finally, signals associated with the hybridized tagged polynucleotide probe are searched for, wherein detected signals being indicative of a chromosome region harboring the human heparanase gene. One ordinarily skilled in the art would know how to use the sequences disclosed herein in suitable labeling reactions and how to use the tagged probes to detect, using *in situ* hybridization, a chromosome region harboring a human heparanase gene.

**[0104]** Reference is now made to the following examples, which together with the above descriptions, illustrate the



invention in a non-limiting fashion.

## EXAMPLES

[0105] The following protocols and experimental details are referenced in the Examples that follow:

**[0106] Purification and characterization of heparanase from a human hepatoma cell line and human placenta:**

A human hepatoma cell line (Sk-hep-1) was chosen as a source for purification of a human tumor-derived heparanase. Purification was essentially as described in U.S. Pat. No. 5,362,641 to Fuks.

[0107] Briefly, 500 liter,  $5 \times 10^{11}$  cells were grown in suspension and the heparanase enzyme was purified about 240,000 fold by applying the following steps: (i) cation exchange (CM-Sephadex) chromatography performed at pH 6.0, 0.3-1.4 M NaCl gradient; (ii) cation exchange (CM-Sephadex) chromatography performed at pH 7.4 in the presence of 0.1% CHAPS, 0.3-1.1 M NaCl gradient; (iii) heparin-Sepharose chromatography performed at pH 7.4 in the presence of 0.1% CHAPS, 0.35-1.1 M NaCl gradient; (iv) ConA-Sepharose chromatography performed at pH 6.0 in buffer containing 0.1% CHAPS and 1 M NaCl, elution with 0.25 M  $\alpha$ -methyl mannoside; and (v) HPLC cation exchange (Mono-S) chromatography performed at pH 7.4 in the presence of 0.1% CHAPS, 0.25-1 M NaCl gradient.

[0108] Active fractions were pooled, precipitated with TCA and the precipitate subjected to SDS polyacrylamide gel electrophoresis and/or tryptic digestion and reverse phase HPLC. Tryptic peptides of the purified protein were separated by reverse phase HPLC (C8 column) and homogeneous peaks were subjected to amino acid sequence analysis.

[0109] The purified enzyme was applied to reverse phase HPLC and subjected to N-terminal amino acid sequencing using the amino acid sequencer (Applied Biosystems).

[0110] **Cells:** Cultures of bovine corneal endothelial cells (BCECs) were established from steer eyes as previously described (19, 38). Stock cultures were maintained in DMEM (1 g glucose/liter) supplemented with 10% newborn calf serum and 5% FCS. bFGF (1 ng/ml) was added every other day during the phase of active cell growth (13, 14).

[0111] **Preparation of dishes coated with ECM:** BCECs (second to fifth passage) were plated into 4-well plates at an initial density of  $2 \times 10^5$  cells/ml, and cultured in sulfate-free Fisher medium plus 5% dextran T-40 for 12 days.  $\text{Na}_2^{35}\text{SO}_4$  (25  $\mu\text{Ci/ml}$ ) was added on day 1 and 5 after seeding and the cultures were incubated with the label without medium change. The subendothelial ECM was exposed by dissolving (5 min., room temperature) the cell layer with PBS containing 0.5% Triton. X-100 and 20 mM  $\text{NH}_4\text{OH}$ , followed by four washes with PBS. The ECM remained intact; free of cellular debris and firmly attached to the entire area of the tissue culture dish (19, 22).

[0112] To prepare soluble sulfate labeled proteoglycans (peak I material), the ECM was digested with trypsin (25  $\mu\text{g/ml}$ , 6 h, 37 °C), the digest was concentrated by reverse dialysis and the concentrated material was applied onto a Sepharose 6B gel filtration column. The resulting high molecular weight material ( $K_{av} < 0.2$ , peak I) was collected. More than 80% of the labeled material was shown to be composed of heparan sulfate proteoglycans (11, 39).

[0113] **Heparanase activity:** Cells ( $1 \times 10^6$ /35-mm dish), cell lysates or conditioned media were incubated on top of  $^{35}\text{S}$ -labeled ECM (18 h, 37 °C) in the presence of 20 mM phosphate buffer (pH 6.2). Cell lysates and conditioned media were also incubated with sulfate labeled peak I material (10-20  $\mu\text{l}$ ). The incubation medium was collected, centrifuged (18,000 x g, 4 °C, 3 min.), and sulfate labeled material analyzed by gel filtration on a Sepharose CL-6B column (0.9 x 30 cm). Fractions (0.2 ml) were eluted with PBS at a flow rate of 5 ml/h and counted for radioactivity using Bio-fluor scintillation fluid. The excluded volume ( $V_0$ ) was marked by blue dextran and the total included volume ( $V_t$ ) by phenol red. The latter was shown to comigrate with free sulfate (7, 11, 23). Degradation fragments of HS side chains were eluted from Sepharose 6B at  $0.5 < K_{av} < 0.8$  (peak II) (7, 11, 23). A nearly intact HSPG released from ECM by trypsin - and, to a lower extent, during incubation with PBS alone - was eluted next to  $V_0$  ( $K_{av} < 0.2$ , peak I). Recoveries of labeled material applied on the columns ranged from 85 to 95% in different experiments (11). Each experiment was performed at least three times and the variation of elution positions ( $K_{av}$  values) did not exceed  $\pm 15\%$ .

[0114] **Cloning of hpa cDNA:** cDNA clones 257548 and 260138 were obtained from the I.M.A.G.E Consortium (2130 Memorial Parkway SW, Huntsville, AL 35801). The cDNAs were originally cloned in *EcoRI* and *NotI* cloning sites in the plasmid vector pT3T7D-Pac. Although these clones are reported to be somewhat different, DNA sequencing demonstrated that these clones are identical to one another. Marathon RACE (RIM) (rapid amplification of cDNA ends) human placenta (poly-A) cDNA composite was a gift of Prof Yossi Shiloh of Tel Aviv University.. This composite is vector free, as it includes reverse transcribed cDNA fragments to which double, partially single stranded adapters are attached on both sides. The construction of the specific composite employed is described in reference 39a.

[0115] Amplification of hp3 PCR fragment was performed according to the protocol provided by Clontech laboratories. The template used for amplification was a sample taken from the above composite. The primers used for amplification were:



First step: 5'-primer: AP1: 5'-CCATCCTAATACGACTCACTATAGGG  
C-3', SEQ ID NO:1; 3'-primer: HPL229: 5'-GTAGTGATGCCATGTAAGTGA  
ATC-3', SEQ ID NO:2.

[0116] Second step: nested 5'-primer AP2: 5'-ACTCACTATAGGGCTCGAGCG GC-3', SEQ ID NO:3; nested 3'- primer: HPL171: 5'-GCATCTTAGCCGTCT TTCTTCG-3', SEQ ID NO:4. The HPL229 and HPL 171 were selected according to the sequence of the EST clones. They include nucleotides 933-956 and 876-897 of SEQ ID NO:9, respectively.

[0117] PCR program was 94 °C - 4 min., followed by 30 cycles of 94 °C - 40 sec., 62 °C - 1 min., 72°C - 2.5 min. Amplification was performed with Expand High Fidelity (Boehringer Mannheim). The resulting ca. 900 bp hp3 PCR product was, digested with *BfrI* and *PvuII*. Clone 257548 (*phpa1*) was digested with *EcoRI*, followed by end filling and was then further digested with *BfrI*. Thereafter the *PvuII* - *BfrI* fragment of the hp3 PCR product was cloned into the blunt end - *BfrI* end of clone *phpa1* which resulted in having the entire cDNA cloned in pT3T7-pac vector, designated *phpa2*.

[0118] **DNA Sequencing:** Sequence determinations were performed with vector specific and gene specific primers, using an automated DNA sequencer (Applied Biosystems, model 373A). Each nucleotide was read from at least two independent primers.

[0119] **Computer analysis of sequences:** Database searches for sequence similarities were performed using the Blast network service. Sequence analysis and alignment of DNA and protein sequences were done using the DNA sequence analysis software package developed by the Genetic Computer Group (GCG) at the University of Wisconsin.

[0120] **RT-PCR:** RNA was prepared using TRI-Reagent (Molecular research center Inc.) according to the manufacturer instructions. 1.25 µg were taken for reverse transcription reaction using MuMLV Reverse transcriptase (Gibco BRL) and Oligo (dT)<sub>15</sub> primer, SEQ ID NO:5, (Promega). Amplification of the resultant first strand cDNA was performed with Taq polymerase (Promega). The following primers were used:

HPU-355: 5'-TTCGATCCCAAGAAGGAATCAAC-3', SEQ ID NO:6, nucleotides 372-394 in SEQ ID NO:9 or 11.

HPL-229: 5'-GTAGTGATGCCATGTAAGTGAATC-3', SEQ ID NO:7, nucleotides 933-956 in SEQ ID NO:9 or 11.

PCR program: 94 °C - 4 min., followed by 30 cycles of 94°C - 40 sec., 62°C - 1 min., 72°C - 1 min.

[0121] **Expression of recombinant heparanase in insect cells:** Cells, High Five (RIM) and Sf21 insect cell lines were maintained as monolayer cultures in SF900II-SFM medium (GibcoBRL).

[0122] **Recombinant Baculovirus:** Recombinant virus containing the *hpa* gene was constructed using the Bac to Bac system (GibcoBRL). The transfer vector pFastBac was digested with *SalI* and *NotI* and ligated with a 1-7 kb fragment of *phpa2* digested with *XhoI* and *NotI*. The resulting plasmid was designated pFast*hpa2*. An identical plasmid designated pFast*hpa4* was prepared as a duplicate and both independently served for further experimentations. Recombinant bacmid was generated according to the instructions of the manufacturer with pFast*hpa2*, pFast*hpa4* and with pFastBac. The latter served as a negative control. Recombinant bacmid DNAs were transfected into Sf21 insect cells. Five days after transfection recombinant viruses were harvested and used to infect High Five (RIM) cells. 3 x 10<sup>6</sup> cells in T-25 flasks. Cells were harvested 2 - 3 days after infection. 4 x 10<sup>6</sup> cells were centrifuged and resuspended in a reaction buffer containing 20 mM phosphate citrate buffer, 50 mM NaCl. Cells underwent three cycles of freeze and thaw and lysates were stored at -80 °C. Conditioned medium was stored at 4 °C.

[0123] **Partial purification of recombinant heparanase:** Partial purification of recombinant heparanase was performed by heparin-Sepharose column chromatography followed by Superdex 75 column gel filtration. Culture medium (150 ml) of Sf21 cells infected with pF*hpa4* virus was subjected to heparin-Sepharose chromatography. Elution of 1 ml fractions was performed with 0.35 - 2 M NaCl gradient in presence of 0.1 % CHAPS and 1 mM DTT in 10 mM sodium acetate buffer, pH 5.0. A 25 µl sample of each fraction was tested for heparanase activity. Heparanase activity was eluted at the range of 0.65 - 1.1 M NaCl (fractions 18-26, Figure 10a). 5 µl of each fraction was subjected to 15 % SDS-polyacrylamide gel electrophoresis followed by silver nitrate staining. Active fractions eluted from heparin-Sepharose (Figure 10a) were pooled and concentrated (x 6) on YM3 cut-off membrane. 0.5 ml of the concentrated material was applied onto 30 ml Superdex 75 FPLC column equilibrated with 10 mM sodium acetate buffer, pH 5.0, containing 0.8 M NaCl, 1 mM DTT and 0.1 % CHAPS. Fractions (0.56 ml) were collected at a flow rate of 0.75 ml/min. Aliquots of each fraction were tested for heparanase activity and were subjected to SDS-polyacrylamide gel electrophoresis followed by silver nitrate staining (Figure 11b).

## EXAMPLE 1

Cloning of the *hpa* gene

[0124] Purified fraction of heparanase isolated from human hepatoma cells (SK-hep-1) was subjected to tryptic digestion and microsequencing. EST (Expressed Sequence Tag) databases were screened for homology to the back translated DNA sequences corresponding to the obtained peptides. Two EST sequences (accession Nos. N41349 and N45367) contained a DNA sequence encoding the peptide YGPDVGQPR (SEQ ID NO:8). These two sequences were derived from clones 257548 and 260138 (I.M.A.G.E Consortium) prepared from 8 to 9 weeks placenta cDNA library (Soares). Both clones which were found to be identical contained an insert of 1020 bp which included an open reading frame (ORF) of 973 bp followed by a 3' untranslated region of 27 bp and a Poly A tail. No translation start site (AUG) was identified at the 5' end of these clones.

[0125] Cloning of the missing 5' end was performed by PCR amplification of DNA from a placenta Marathon RACE (RIM) cDNA composite. A 900 bp fragment (designated hp3), partially overlapping with the identified 3' encoding EST clones was obtained

[0126] The joined cDNA fragment, 1721 bp long (SEQ ID NO:9), contained an open reading frame which encodes, as shown in Figure 1 and SEQ ID NO: 11, a polypeptide of 543 amino acids (SEQ ID NO:10) with a calculated molecular weight of 61,192 daltons. The 3' end of the partial cDNA inserts contained in clones 257548 and 260138 started, at nucleotide G<sup>721</sup> of SEQ ID NO:9 and Figure 1.

[0127] As further shown in Figure 1, there was a single sequence discrepancy between the EST clones and the PCR amplified sequence, which led to an amino acid substitution from Tyr<sup>246</sup> in the EST to Phe<sup>246</sup> in the amplified cDNA. The nucleotide sequence of the PCR amplified cDNA fragment was verified from two independent amplification products. The new gene was designated *hpa*.

[0128] As stated above, the 3' end of the partial cDNA inserts contained in EST clones 257548 and 260138 started at nucleotide 721 of *hpa* (SEQ ID NO:9). The ability of the *hpa* cDNA to form stable secondary structures, such as stem and loop structures involving nucleotide stretches in the vicinity of position 721 was investigated using computer modeling. It was found that stable stem and loop structures are likely to be formed involving nucleotides 698-724 (SEQ ID NO:9). In addition, a high GC content, up to 70 %, characterizes the 5' end region of the *hpa* gene, as compared to about only 40 % in the 3' region. These findings may explain the immature termination and therefore lack of 5' ends in the EST clones.

[0129] To examine the ability of the *hpa* gene product to catalyze degradation of heparan sulfate in an *in vitro* assay the entire open reading frame was expressed in insect cells, using the Baculovirus expression system. Extracts of cells, infected with virus containing the *hpa* gene, demonstrated a high level of heparan sulfate degradation activity, while cells infected with a similar construct containing no *hpa* gene had no such activity, nor did non-infected cells. These results are further demonstrated in the following Examples.

## EXAMPLE 2

## Degradation of soluble ECM-derived HSPG

[0130] Monolayer cultures of High Five (RIM) cells were infected (72 h, 28 °C) with recombinant Baculovirus containing the pFast*hpa* plasmid or with control virus containing an insert free plasmid. The cells were harvested and lysed in heparanase reaction buffer by three cycles of freezing and thawing. The cell lysates were then incubated (18 h, 37 °C) with sulfate labeled, ECM-derived HSPG (peak 1), followed by gel filtration analysis (Sephacrose 6B) of the reaction mixture.

[0131] As shown in Figure 2, the substrate alone included almost entirely high molecular weight (Mr) material eluted next to V<sub>0</sub> (peak I, fractions 5-20, K<sub>av</sub> < 0.35). A similar elution pattern was obtained when the HSPG substrate was incubated with lysates of cells that were infected with control virus. In contrast, incubation of the HSPG substrate with lysates of cells infected with the *hpa* containing virus resulted in a complete conversion of the high Mr substrate into low Mr labeled degradation fragments (peak II, fractions 22-35, 0.5 < K<sub>av</sub> < 0.75).

[0132] Fragments eluted in peak II were shown to be degradation products of heparan sulfate, as they were (i) 5- to 6-fold smaller than intact heparan sulfate side chains (K<sub>av</sub> approx. 0.33) released from ECM by treatment with either alkaline borohydride or papain; and (ii) resistant to further digestion with papain or chondroitinase ABC, and susceptible to deamination by nitrous acid (6, 11).

[0133] Similar results (not shown) were obtained with Sf21 cells. Again, heparanase activity was detected in cells infected with the *hpa* containing virus (pF*hpa*), but not with control virus (pF). This result was obtained with two independently generated recombinant viruses. Lysates of control not infected High Five (RIM) cells failed to degrade the HSPG substrate.

[0134] In subsequent experiments, the labeled HSPG substrate was incubated with medium conditioned by infected

High Five (RIM) or Sf21 cells.

[0135] As shown in Figures 3a-b, heparanase activity, reflected by the conversion of the high Mr peak I substrate into the low Mr peak II which represents HS degradation fragments, was found in the culture medium of cells infected with the pF*hpa2* or pF*hpa4* viruses, but not with the control pF1 or pF2 viruses. No heparanase activity was detected in the culture medium of control non-infected High Five (RIM) or Sf21 cells.

[0136] The medium of cells infected with the pF*hpa4* virus was passed through a 50 kDa cut off membrane to obtain a crude estimation of the molecular weight of the recombinant heparanase enzyme. As demonstrated in Figure 4, all the enzymatic activity was retained in the upper compartment and there was no activity in the flow through (<50 kDa) material. This result is consistent with the expected molecular weight of the *hpa* gene product.

[0137] In order to further characterize the *hpa* product the inhibitory effect of heparin, a potent inhibitor of heparanase mediated HS degradation (40) was examined.

[0138] As demonstrated in Figures 5a-b, conversion of the peak I substrate into peak II HS degradation fragments was completely abolished in the presence of heparin.

[0139] Altogether, these results indicate that the heparanase enzyme is expressed in an active form by insect cells infected with Baculovirus containing the newly identified human *hpa* gene.

### EXAMPLE 3

#### Degradation of HSPG in intact ECM

[0140] Next, the ability of intact infected insect cells to degrade HS in intact, naturally produced ECM was investigated. For this purpose, High Five (RIM) or Sf21 cells were seeded on metabolically sulfate labeled ECM followed by infection (48 h, 28 °C) with either the pF*hpa4* or control pF2 viruses. The pH of the medium was then adjusted to pH 6.2-6.4 and the cells further incubated with the labeled ECM for another 48 h at 28 °C or 24 h at 37 °C. Sulfate labeled material released into the incubation medium was analyzed by gel filtration on Sepharose 6B.

[0141] As shown in Figures- 6a-b and 7a-b, incubation of the ECM with cells infected with the control pF2 virus resulted in a constant release of labeled material that consisted almost entirely (>90%) of high Mr fragments (peak I) eluted with or next to  $V_0$ . It was previously shown that a proteolytic activity residing in the ECM itself and/or expressed by cells is responsible for release of the high Mr material (6). This nearly intact HSPG provides a soluble substrate for subsequent degradation by heparanase, as also indicated by the relatively large amount of peak I material accumulating when the heparanase enzyme is inhibited by heparin (6, 7, 12, Figure 9). On the other hand, incubation of the labeled ECM with cells infected with the pF*hpa4* virus resulted in release of 60-70% of the ECM-associated radioactivity in the form of low Mr sulfate-labeled fragments (peak II,  $0.5 < K_{av} < 0.75$ ), regardless of whether the infected cells were incubated with the ECM at 28 °C or 37 °C. Control intact non-infected Sf21 or High Five (RIM) cells failed to degrade the ECM HS side chains.

[0142] In subsequent experiments, as demonstrated in Figs. 8a-b, High Five (RIM) and Sf21 cells were infected (96 h, 28 °C) with pF*hpa4* or control pF1 viruses and the culture medium incubated with sulfate-labeled ECM. Low Mr HS degradation fragments were released from the ECM only upon incubation with medium conditioned by pF*hpa4* infected cells. As shown in Figure 9, production of these fragments was abolished in the presence of heparin. No heparanase activity was detected in the culture medium of control, non-infected cells. These results indicate that the heparanase enzyme expressed by cells infected with the pF*hpa4* virus is capable of degrading HS when complexed to other macromolecular constituents (i.e. fibronectin, laminin, collagen) of a naturally produced intact ECM, in a manner similar to that reported for highly metastatic tumor cells or activated cells of the immune system (6; 7).

### EXAMPLE 4

#### Purification of recombinant heparanase

[0143] The recombinant heparanase was partially purified from medium of pF*hpa4* infected Sf21 cells by Heparin-Sepharose chromatography (Figure 10a) followed by gel filtration of the pooled active fractions over an FPLC Superdex 75 column (Figure 11a). A ~ 63 kDa protein was observed, whose quantity, as was detected by silver stained SDS-polyacrylamide gel electrophoresis, correlated with heparanase activity in the relevant column fractions (Figures 10b and 11b, respectively). This protein was not detected in the culture medium of cells infected with the control pF1 virus and was subjected to a similar fractionation on heparin-Sepharose (not shown).

**EXAMPLE 5****Expression of the *hpa* gene in various cell types, organs and tissues**

[0144] Referring now to Figures 12a-e, RT-PCR was applied to evaluate the expression of the *hpa* gene by various cell types and tissues. For this purpose, total RNA was reverse transcribed and amplified. The expected 585 bp long cDNA was clearly demonstrated in human kidney, placenta (8 and 11 weeks) and mole tissues, as well as in freshly isolated and short termed (1.5-48 h) cultured human placental cytotrophoblastic cells (Figure 12a), all known to express a high heparanase activity (41). The *hpa* transcript was also expressed by normal human neutrophils (Figure 12b). In contrast, there was no detectable expression of the *hpa* mRNA in embryonic human muscle tissue, thymus, heart and adrenal. (Figure 12b). The *hpa* gene was expressed by several, but not all, human bladder carcinoma cell lines (Figure 12c), SK hepatoma (SK-hep-1), ovarian carcinoma (OV 1063), breast carcinoma (435, 231), melanoma and megakaryocytic (DAMI, CHRF) human cell lines (Figures 12d-e).

[0145] The above described expression pattern of the *hpa* transcript was determined to be in a very good correlation with heparanase activity levels determined in various tissues and cell types (not shown).

**EXAMPLE 6*****hpa* homologous genes**

[0146] EST databases were screened for sequences homologous to the *hpa* gene. Three mouse ESTs were identified (accession No. Aa177901, from mouse spleen, Aa067997 from mouse skin, Aa47943 from mouse embryo), assembled into a 824 bp cDNA fragment which contains a partial open reading frame (lacking a 5' end) of 629 bp and a 3' untranslated region of 195 bp (SEQ ID NO:12). As shown in Figure 13, the coding region is 80% similar to the 3' end of the *hpa* cDNA sequence. These ESTs are probably cDNA fragments of the mouse *hpa* homolog that encodes for the mouse heparanase.

[0147] Searching for consensus protein domains revealed an amino terminal homology between the heparanase and several precursor proteins such as Procollagen Alpha 1 precursor, Tyrosine-protein kinase-RYK, Fibulin-1, Insulin-like growth factor binding protein and several others. The amino terminus is highly hydrophobic and contains a potential trans-membrane domain. The homology to known signal peptide sequences suggests that it could function as a signal peptide for protein localization.

**EXAMPLE 7*****Isolation of an extended 5' end of hpa cDNA from human SK-hep1 cell line***

[0148] The 5' end of *hpa* cDNA was isolated from human SK-hep1 cell line by PCR amplification using the Marathon RACE (RIM) (rapid amplification of cDNA ends) kit (Clontech). Total RNA was prepared from SK-hep1 cells using the TRI-Reagent (Molecular research center Inc.) according to the manufacturer instructions. Poly A+ RNA was isolated using the mRNA separator kit (Clontech).

[0149] The Marathon RACE SK-hep1 cDNA composite was constructed according to the manufacturer recommendations. First round of amplification was performed using an adaptor specific primer AP1: 5'-CCATCCTAATACG ACT-CACTATAGGGC-3', SEQ ID NO:1, and a *hpa* specific antisense primer hpl-629: 5'-CCCCAGGAGCAGCAGCATCAG-3', SEQ ID NO:17, corresponding to nucleotides 119-99 of SEQ ID NO:9. The resulting PCR product was subjected to a second round of amplification using an adaptor specific nested primer AP2: 5'-ACTCACTATAGGGCTCGAGCGGC-3', SEQ ID NO:3, and a *hpa* specific antisense nested primer hpl-666 5'-AGGCTTCGAGCGCAGCAGCAT-3', SEQ ID NO:18, corresponding to nucleotides 83-63 of SEQ ID NO:9. The PCR program was as follows: a hot start of 94 °C for 1 minute, followed by 30 cycles of 90 °C - 30 seconds, 68 °C - 4 minutes. The resulting 300 bp DNA fragment was extracted from an agarose gel and cloned into the vector pGEM-T Easy (Promega). The resulting recombinant plasmid was designated pHPSK1.

[0150] The nucleotide sequence of the pHPSK1 insert was determined and it was found to contain 62 nucleotides of the 5' end of the placenta *hpa* cDNA (SEQ ID NO:9) and additional 178 nucleotides upstream, the first 178 nucleotides of SEQ ID NOs:13 and 15.

[0151] A single nucleotide discrepancy was identified between the SK-hep1 cDNA and the placenta cDNA. The "T" derivative at position 9 of the placenta cDNA (SEQ ID NO:9), is replaced by a "C" derivative at the corresponding position 187 of the SK-hep cDNA (SEQ ID NO: 13).

[0152] The discrepancy is likely to be due to a mutation at the 5' end of the placenta cDNA clone as confirmed by sequence analysis of several additional cDNA clones isolated from placenta, which like the SK-hep1 cDNA contained C at position 9 of SEQ ID NO:9..



[0153] The 5' extended sequence of the SK-hepl *hpa* cDNA was assembled with the sequence of the *hpa* cDNA isolated from human placenta (SEQ ID NO:9). The assembled sequence contained an open reading frame which encodes, as shown in SEQ ID NOs:14 and 15, a polypeptide of 592 amino acids with a calculated molecular weight of 66,407 daltons. The open reading frame is flanked by 93 bp 5' untranslated region (UTR).

## EXAMPLE 8

### *Isolation of the upstream genomic region of the hpa gene*

[0154] The upstream region of the *hpa* gene was isolated using the Genome Walker kit (Clontech) according to the manufacturer recommendations. The kit includes five human genomic DNA samples each digested with a different restriction endonuclease creating blunt ends: *EcoRV*, *ScaI*, *DraI*, *PvuII* and *SspI*.

[0155] The blunt ended DNA fragments are ligated to partially single stranded adaptors. The Genomic DNA samples were subjected to PCR amplification using the adaptor specific primer and a gene specific primer. Amplification was performed with Expand High Fidelity (Boehringer Mannheim).

[0156] A first round of amplification was performed using the ap1 primer: 5'-G TAATACGACTCACTATAGGGC-3', SEQ ID NO:19, and the *hpa* specific antisense primer hpl-666: 5'-AGGCTTCGAGCGCAGCAGCAT-3', SEQ ID NO:18, corresponding to nucleotides 83—63 of SEQ ID NO:9. The PCR program was as follows: a hot start of 94 °C - 3 minutes, followed by 36 cycles of 94 °C - 40 seconds, 67 °C - 4 minutes.

[0157] The PCR products of the first amplification were diluted 1:50. One µl of the diluted sample was used as a template for a second amplification using a nested adaptor specific primer ap2: 5'-ACTATAGGGCACGCGTGGT-3', SEQ ID NO:20, and a *hpa* specific antisense primer hpl-690, 5'-CTTGGGCTCACC TGGCTGCTC-3', SEQ ID NO:21, corresponding to nucleotides 62-42 of SEQ ID NO:9. The resulting amplification products were analyzed using agarose gel electrophoresis. Five different PCR products were obtained from the five amplification reactions. A DNA fragment of approximately 750 bp which was obtained from the *SspI* digested DNA sample was gel extracted. The purified fragment was ligated into the plasmid vector pGEM-T Easy (Promega). The resulting recombinant plasmid was designated pGHP6905 and the nucleotide sequence of the *hpa* insert was determined.

[0158] A partial sequence of 594 nucleotides is shown in SEQ ID NO:16. The last nucleotide in SEQ ID NO:13 corresponds to nucleotide 93 in SEQ ID:13. The DNA sequence in SEQ ID NO:16 contains the 5' region of the *hpa* cDNA and 501 nucleotides of the genomic upstream region which are predicted to contain the promoter region of the *hpa* gene.

## EXAMPLE 9

### *Expression of the 592 amino acids HPA polypeptide in a human 293 cell line*

[0159] The 592 amino acids open reading frame (SEQ ID NOs:13 and 15) was constructed by ligation of the 110 bp corresponding to the 5' end of the SK-hep 1 *hpa* cDNA with the placenta cDNA. More specifically the Marathon RACE (RIM) PCR amplification product of the placenta *hpa* DNA was digested with *SacI* and an approximately 1 kb fragment was ligated into a *SacI*-digested pGHP6905 plasmid. The resulting plasmid was digested with *EarI* and *AatII*. The *EarI* sticky ends were blunted and an approximately 280 bp *EarI*/blunt-*AatII* fragment was isolated. This fragment was ligated with pFast*hpa* digested with *EcoRI* which was blunt ended using Klenow fragment and further digested with *AatII*. The resulting plasmid contained a 1827 bp insert which includes an open reading frame of 1776 bp, 31 bp of 3' UTR and 21 bp of 5' UTR. This plasmid was designated pFastL*hpa*.

[0160] A mammalian expression vector was constructed to drive the expression of the 592 amino acids heparanase polypeptide in human cells. The *hpa* cDNA was excised from pFastL*hpa* with *BssHII* and *NofI*. The resulting 1850 bp *BssHII*-*NofI* fragment was ligated to a mammalian expression vector pSI (Promega) digested with *MluI* and *NofI*. The resulting recombinant plasmid, pSI*hpa*Met2 was transfected into a human 293 embryonic kidney cell line.

[0161] Transient expression of the 592 amino-acids heparanase was examined by western blot analysis and the enzymatic activity was tested using the gel shift assay. Both these procedures are described in length in U.S. Pat. application No. 09/071,739, filed May 1, 1998, which is incorporated by reference as if fully set forth herein. Cells were harvested 3 days following transfection. Harvested cells were re-suspended in lysis buffer containing 150 mM NaCl, 50 mM Tris pH 7.5, 1% Triton X-100, 1 mM PMSF and protease inhibitor cocktail (Boehringer Mannheim). 40 µg protein extract samples were used for separation on a SDS-PAGE. Proteins were transferred onto a PVDF Hybond-P membrane (Amersham). The membrane was incubated with an affinity purified polyclonal anti heparanase antibody, as described in U.S. Pat. application No. 09/071,739. A major band of approximately 50 kDa was observed in the transfected cells as well as a minor band of approximately 65 kDa. A similar pattern was observed in extracts of cells transfected with the p*Shpa* as demonstrated in U.S. Pat application No. 09/071,739. These two bands probably represent two forms of

the recombinant heparanase protein produced by the transfected cells. The 65 kDa protein probably represents a heparanase precursor, while the 50 kDa protein is suggested herein to be the processed or mature form.

[0162] The catalytic activity of the recombinant protein expressed in the pShpaMet2 transfected cells was tested by gel shift assay. Cell extracts of transfected and of mock transfected cells were incubated overnight with heparin (6 µg in each reaction) at 37 °C, in the presence of 20 mM phosphate citrate buffer pH 5.4, 1 mM CaCl<sub>2</sub>, 1 mM DTT and 50 mM NaCl. Reaction mixtures were then separated on a 10 % polyacrylamide gel. The catalytic activity of the recombinant heparanase was clearly demonstrated by a faster migration of the heparin molecules incubated with the transfected cell extract as compared to the control. Faster migration indicates the disappearance of high molecular weight heparin molecules and the generation of low molecular weight degradation products.

## EXAMPLE 10

### *Chromosomal localization of the hpa gene*

[0163] Chromosomal mapping of the *hpa* gene was performed utilizing a panel of monochromosomal human/CHO and human/mouse somatic cell hybrids, obtained from the UK HGMP Resource Center (Cambridge, England).

[0164] 40 ng of each of the somatic cell hybrid DNA samples were subjected to PCR amplification using the *hpa* primers: hpu565 5'-AGCTCTGTAGATGTGC TATACAC-3', SEQ ID NO:22, corresponding to nucleotides 564-586 of SEQ ID NO:9 and an antisense primer hpl171 5'-GCATCTTAGCCGTCTTTCTTCG-3', SEQ ID NO:23, corresponding to nucleotides 897-876 of SEQ ID NO:9.

[0165] The PCR program was as follows: a hot start of 94 °C - 3 minutes, - followed by 7 cycles of 94 °C - 45 seconds, 66 °C - 1 minute, 68 °C - 5 minutes, followed by 30 cycles of 94 °C - 45 seconds, 62°C - 1 minute, 68°C - 5 minutes, and a 10 minutes final extension at 72°C.

[0166] The reactions were performed with Expand long PCR (Boehringer Mannheim). The resulting amplification products were analyzed using agarose gel electrophoresis. As demonstrated in Figure 14, a single band of approximately 2.8 Kb was obtained from chromosome 4, as well as from the control human genomic DNA. A 2.8 kb amplification product is expected based on amplification of the genomic *hpa* clone (data not shown). No amplification products were obtained neither in the control DNA samples of hamster and mouse nor in somatic hybrids of other human chromosome.

## LIST OF REFERENCES CITED HEREINABOVE BY NUMBERS

### [0167]

1. Wight, T.N., Kinsella, M.G., and Qwarnstromn, E.E. (1992). The role of proteoglycans in cell adhesion, migration and proliferation. *Curr. Opin. Cell Biol.*, **4**, 793-801.

2. Jackson, R.L., Busch, S.J., and Cardin, A.L. (1991). Glycosaminoglycans: Molecular properties, protein interactions and role in physiological processes. *Physiol. Rev.*, **71**, 481-539.

3. Wight, T.N. (1989). Cell biology of arterial proteoglycans. *Arteriosclerosis*, **9**, 1-20.

4. Kjellen, L., and Lindahl, U. (1991). Proteoglycans: structures and interactions. *Annu. Rev. Biochem.*, **60**, 443-475.

5. Ruoslahti, E., and Yamaguchi, Y. (1991). Proteoglycans as modulators of growth factor activities. *Cell*, **64**, 867-869.

6. Vlodavsky, I., Eldor, A., Haimovitz-Friedman, A., Matzner, Y., Ishai-Michaeli, R., Levi, E., Bashkin, P., Lider, O., Naparstek, Y., Cohen, I.R., and Fuks, Z. (1992). Expression of heparanase by platelets and circulating cells of the immune system: Possible involvement in diapedesis and extravasation. *Invasion & Metastasis*, **12**, 112-127.

7. Vlodavsky, I., Mohsen, M., Lider, O., Ishai-Michaeli, R., Ekre, H.-P., Svahn, C.M., Vigoda, M., and Peretz, T. (1995). Inhibition of tumor metastasis by heparanase inhibiting species of heparin. *Invasion & Metastasis*, **14**, 290-302.

8. Nakajima, M., Irimura, T., and Nicolson, G.L. (1988). Heparanase and tumor metastasis. *J. Cell. Biochem.*, **36**, 157-167.

9. Nicolson, G.L. (1988). Organ specificity of tumor metastasis: Role of preferential adhesion, invasion and growth of malignant cells at specific secondary sites. *Cancer Met. Rev.*, **7**, 143-188.

10. Liotta, L.A., Rao, C.N., and Barsky, S.H. (1983). Tumor invasion and the extracellular matrix. *Lab. Invest.*, **49**, 639-649.

11. Vlodavsky, I., Fuks, Z., Bar-Ner, M., Ariav, Y., and Schirmacher, V. (1983). Lymphoma cell mediated degradation of sulfated proteoglycans in the subendothelial extracellular matrix: Relationship to tumor cell metastasis. *Cancer Res.*, **43**, 2704-2711.

12. Vlodavsky, I., Ishai-Michaeli, R., Bar-Ner, M., Fridman, R., Horowitz, A.T., Fuks, Z. and Biran, S. (1988). Involvement of heparanase in tumor metastasis and angiogenesis. *Is. J. Med.*, **24**, 464-470.

13. Vlodavsky, I., Liu, G.M., and Gospodarowicz, D. (1980). Morphological appearance, growth behavior and migratory activity of human tumor cells maintained on extracellular matrix vs. plastic. *Cell*, **19**, 607-616.

14. Gospodarowicz, D., Delgado, D., and Vlodavsky, I. (1980). Permissive effect of the extracellular matrix on cell proliferation in-vitro. *Proc. Natl. Acad. Sci. USA.*, **77**, 4094-4098.

15. Bashkin, P., Doctrow, S., Klagsbrun, M., Svahn, C.M., Folkman, J., and Vlodavsky, I. (1989). Basic fibroblast growth factor binds to subendothelial extracellular matrix and is released by heparitinase and heparin-like molecules. *Biochemistry*, **28**, 1737-1743.

16. Parish, C.R., Coombe, D.R., Jakobsen, K.B., and Underwood, P.A. (1987). Evidence that sulphated polysaccharides inhibit tumor metastasis by blocking tumor cell-derived heparanase. *Int. J. Cancer*, **40**, 511-517.

16a. Vlodavsky, I., Hua-Quan Miao., Benezra, M., Lider, O., Bar-Shavit, R., Schmidt, A., and Peretz, T. (1997). Involvement of the extracellular matrix, heparan sulfate proteoglycans and heparan sulfate degrading enzymes in angiogenesis and metastasis. In: Tumor Angiogenesis. Eds. C.E. Lewis, R. Bicknell & N. Ferrara. Oxford University Press, Oxford UK, pp. 125-140.

17. Burgess, W.H., and Maciag, T. (1989). The heparin-binding (fibroblast) growth factor family of proteins. *Annu. Rev. Biochem.*, **58**, 575-606.

18. Folkman, J., and Klagsbrun, M. (1987). Angiogenic factors. *Science*, **235**, 442-447.

19. Vlodavsky, I., Folkman, J., Sullivan, R., Fridman, R., Ishai-Michaeli, R., Sasse, J., and Klagsbrun, M. (1987). Endothelial cell-derived basic fibroblast growth factor: Synthesis and deposition into subendothelial extracellular matrix. *Proc. Natl. Acad. Sci. USA*, **84**, 2292-2296.

20. Folkman, J., Klagsbrun, M., Sasse, J., Wadzinski, M., Ingber, D., and Vlodavsky, I. (1980). A heparin-binding angiogenic protein - basic fibroblast growth factor - is stored within basement membrane. *Am. J Pathol.*, **130**, 393-400.

21. Cardon-Cardo, C., Vlodavsky, I., Haimovitz-Friedman, A., Hicklin, D., and Fuks, Z. (1990). Expression of basic fibroblast growth factor in normal human tissues. *Lab. Invest.*, **63**, 832-840.

22. Ishai-Michaeli, R., Svahn, C.-M., Chajek-Shaul, T., Komer, G., Ekre, H.-P., and Vlodavsky, I. (1992). Importance of size and sulfation of heparin in release of basic fibroblast factor from the vascular endothelium and extracellular matrix. *Biochemistry*, **31**, 2080-2088.

23. Ishai-Michaeli, R., Eldor, A., and Vlodavsky, I. (1990). Heparanase activity expressed by platelets, neutrophils and lymphoma cells releases active fibroblast growth factor from extracellular matrix. *Cell Reg.*, **1**, 833-842.

24. Vlodavsky, I., Bar-Shavit, R., Ishai-Michaeli, R., Bashkin, P., and Fuks, Z. (1991). Extracellular sequestration and release of fibroblast growth factor: a regulatory mechanism? *Trends Biochem. Sci.*, **16**, 268-271.

25. Vlodavsky, I., Bar-Shavit, R., Komer, G., and Fuks, Z. (1993). Extracellular matrix-bound growth factors, enzymes and plasma proteins. In Basement membranes: Cellular and molecular aspects (eds. D.H. Rohrbach and R. Timpl), pp327-343. Academic press Inc., Orlando, FL.

26. Yayon, A., Klagsbrun, M., Esko, J.D., Leder, P., and Omitz, D.M. (1991). Cell surface, heparin-like molecules

are required for binding of basic fibroblast growth factor to its high affinity receptor. *Cell*, **64**, 841-848.

27. Spivak-Kroizman, T., Lemmon, M.A., Dikic, I., Ladbury, J.E., Pinchasi, D., Huang, J., Jaye, M., Crumley, G., Schlessinger, J., and Lax, I. (1994). Heparin-induced oligomerization of FGF molecules is responsible for FGF receptor dimerization, activation, and cell proliferation. *Cell*, **79**, 1015-1024.

28. Omitz, D.M., Herr, A.B., Nilsson, M., West, a., J., Svahn, C.-M., and Waksman, G. (1995). FGF binding and FGF receptor activation by synthetic heparan-derived di- and trisaccharides. *Science*, **268**, 432-436.

29. Gitay-Goren, H., Soker, S., Vlodavsky, I., and Neufeld, G. (1992). Cell surface associated heparin-like molecules are required for the binding of vascular endothelial growth factor (VEGF) to its cell surface receptors. *J Biol. Chem.*, **267**, 6093-6098.

30. Lider, O., Baharav, E., Mekori, Y., Miller, T., Naparstek, Y., Vlodavsky, I., and Cohen, I.R. (1989). Suppression of experimental autoimmune diseases and prolongation of allograft survival by treatment of animals with heparinoid inhibitors of T lymphocyte heparanase. *J. Clin. Invest.*, **83**, 752-756.

31. Lider, O., Cahalon, L., Gilat, D., HersHKovitz, R., Siegel, D, Margalit, R., Shoseyov, O., and Cohn, I.R. (1995). A disaccharide that inhibits tumor necrosis factor  $\alpha$  is formed from the extracellular matrix by the enzyme heparanase. *Proc. Natl. Acad. Sci. USA.*, **92**, 5037-5041.

31a. Rapraeger, A., Krufica, A., and Olwin, B.R. (1991). Requirement of heparan sulfate for bFGF-mediated fibroblast growth and myoblast differentiation. *Science*, **252**, 1705-1708.

32. Eisenberg, S., Sehayek, E., Olivecrona, T., and Vlodavsky, I. (1992). Lipoprotein lipase enhances binding of lipoproteins to heparan sulfate on cell surfaces and extracellular matrix. *J. Clin. Invest.*, **90**, 2013-2021.

33. Shieh, M-T., Wundunn, D., Montgomery, R.I, Esko, J.D., and Spear, P.G. J. (1992). Cell surface receptors for herpes simplex virus are heparan sulfate proteoglycans. *J Cell Biol.*, **116**, 1273-1281.

33a. Chen, Y., Maguire, T., Hileman, R.E., Fromm, J.R., Esko, J.D., Linhardt, R.J., and Marks, R.M. (1997). Dengue virus infectivity depends on envelope protein binding to target cell heparan sulfate. *Nature Medicine* **3**, 866-871.

33b. Putnak, J.R., Kanesa-Thanan, N., and Innis, B.L. (1997). A putative cellular receptor for dengue viruses. *Nature Medicine* **3**, 828-829.

34. Narindrasorasak, S., Lowery, D., Gonzalez-DeWhitt, P., Poorman, R.A., Greenberg, B., Kisilevsky, R. (1991). High affinity interactions between the Alzheimer's beta-amyloid precursor protein and the basement membrane form of theparan sulfate proteoglycan. *J. Biol. Chem.*, **266**, 12878-83.

35. Ross, R. (1993). The pathogenesis of atherosclerosis: a perspective for the 1990s. *Nature (Lond.)*, **362**:801-809.

36. Zhong-Sheng, J., Walter, J., Brecht, R., Miranda, D., Mahmood Hussain, M., Innerarity, T.L. and Mahley, W.R. (1993). Role of heparan sulfate proteoglycans in the binding and uptake of apolipoprotein E-enriched remnant lipoproteins by cultured cells. *J. Biol. Chem.*, **268**, 10160-10167.

37. Ernst, S., Langer, R., Cooney, Ch.L., and Sasisekharan, R. (1995). Enzymatic degradation of glycosaminoglycans. *Critical Reviews in Biochemistry and Molecular Biology*, **30**(5), 387-444.

38. Gospodarowicz, D., Mescher, AL., Birdwell, CR. (1977). Stimulation of corneal endothelial cell proliferation in vitro by fibroblast and epidermal growth factors. *Exp Eye Res* **25**, 75-89.

39. Haimovitz-Friedman, A., Falcone, D.J., Eldor, A., Schimnacher, V., Vlodavsky, I., and Fuks, Z. (1991) Activation of platelet heparitinase by tumor cell-derived factors. *Blood*, **78**, 789-796.

39a. Savitsky, K., Platzer, M., Uziel, T., Gilad, S., Sartiel, A., Rosental, A., Elroy-Stein, O., Siloh, Y. and Rotman, G. (1997). Ataxia-telangiectasia: structural diversity of untranslated sequences suggests complex post-translational regulation of ATM gene expression. *Nucleic Acids Res.* **25**(9), 1678-1684.



40. Bar-Ner, M., Eldor, A., Wasserman, L., Matzner, Y., and Vlodavsky, I. (1987). Inhibition of heparanase mediated degradation of extracellular matrix heparan sulfate by modified and non-anticoagulant heparin species. *Blood*, **70**, 551-557.
- 5 41. Goshen, R., Hochberg, A., Korner, G., Levi, E., Ishai- Michaeli, R., Elkin, M., de Grot, N., and Vlodavsky, I. (1996). Purification and characterization of placental heparanase and its expression by cultured cytotrophoblasts. *Mol. Human Reprod.* **2**, 679-684.

SEQUENCE LISTING

10 [0168]

(1) GENERAL INFORMATION:

- 15 (i) APPLICANT: Iris Pecker, Israel Vlodavsky and Elena Feinstein  
 (ii) TITLE OF INVENTION: POLYNUCLEOTIDE ENCODING A POLYPEPTIDE HAVING HEPARANASE ACTIVITY AND EXPRESSION OF SAME IN TRANSDUCED CELLS  
 (iii) NUMBER OF SEQUENCES: 23  
 (iv) CORRESPONDENCE ADDRESS:

- 20 (A) ADDRESSEE: Mark M. Friedman c/o Robert Sheinbein  
 (B) STREET: 2940 Birchtree lane  
 (C) CITY: Silver Spring  
 (D) STATE: Maryland  
 25 (E) COUNTRY: United States of America  
 (F) ZIP: 20906

(v) COMPUTER READABLE FORM:

- 30 (A) MEDIUM TYPE: 1.44 megabyte, 3.5" microdisk  
 (B) COMPUTER: Twinhead\* Slimnote-890TX  
 (C) OPERATING SYSTEM: MS DOS version 6.2, Windows version 3.11  
 (D) SOFTWARE: Word for Windows version 2.0 converted to an ASCII file

35 (vi) CURRENT APPLICATION DATA:

- (A) APPLICATION NUMBER:  
 (B) FILING DATE:  
 40 (C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:

- (A) APPLICATION NUMBER: 08/922,170  
 45 (B) FILING DATE: 2 SEP 1997

(viii) ATTORNEY/AGENT INFORMATION:

- 50 (A) NAME: Friedmam, Mark M.  
 (B) REGISTRATION NUMBER: 33,883  
 (C) REFERENCE/DOCKET NUMBER: 910/1

(ix) TELECOMMUNICATION INFORMATION:

- 55 (A) TELEPHONE: 972-3-5625553  
 (B) TELEFAX: 972-3-5625554  
 (C) TELEX:

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 27  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D.) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

CCATCCTAAT ACGACTCACT ATAGGGC 27

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 24  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

GTAGTGATGC CATGTAAC TG AATC 24

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 23  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

ACTCACTATA GGGCTCGAGC GGC 23

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 22  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

GCATCTTAGC CGTCTTCTT CG 22

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 15
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

5

(xi) SEQUENCE DESCRIPTION: SEQ 10 NO:5:

TTTTTTTTTT TTTT 15

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(2) INFORMATION FOR SEQ 10 NO:6:

(i) SEQUENCE CHARACTERISTICS:

15

- (A) LENGTH: 23
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

TTCGATCCCA AGAAGGAATC AAC 23

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(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

30

- (A) LENGTH: 24
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

35

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

GTAGTGATGC CATGTAAC TG AATC 24

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(2) INFORMATION FOR SEQ 10 NO:8:

(i) SEQUENCE CHARACTERISTICS:

45

- (A) LENGTH: 9
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

50

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Tyr Gly Pro Asp Val Gly Gln Pro Arg  
5 9

(2) INFORMATION FOR SEQ ID NO:9:

55

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1721
- (B) TYPE: nucleic acid

- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

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CTAGAGCTTT CGAGTCTCCG CTGCGCGGCA GCTGGCGGGG GGAGCAGCCA GGTGAGCCCA 60  
AGATGCTGCT GCGCTCGAAG CCTGCGCTGC CGCCGCCGCT GATGCTGCTG CTCCTGGGGC 120  
CGCTGGGTCC CCTCTCCCCT GCGCGCCCTGC CCCGACCTGC GCAAGCACAG GACGTCGTGG 180  
ACCTGGACTT CTTACCCAG GAGCCGCTGC ACCTGGTGAG CCCCTCGTTC CTGTCCGTCA 240  
CCATTGACGC CAACCTGGCC ACGGACCCGC GGTTCCTCAT CCTCCTGGGT TCTCCAAAGC 300  
TTCGTACCTT GGCCAGAGGC TTGTCTCCTG CGTACCTGAG GTTTGGTGGC ACCAAGACAG 360  
ACTTCCTAAT TTTGATCCC AAGAAGGAAT CAACCTTTGA AGAGAGAAGT TACTGGCAAT 420  
CTCAAGTCAA CCAGGATATT TGCAAATATG GATCCATCCC TCCTGATGTG GAGGAGAAGT 480  
TACGGTTGGA ATGGCCCTAC CAGGAGCAAT TGCTACTCCG AGAACACTAC CAGAAAAAGT 540  
TCAAGAACAG CACCTACTCA AGAAGCTCTG TAGATGTGCT ATACACTTTT GCAAACTGCT 600  
CAGGACTGGA CTTGATCTTT GGCCATAATG CGTTATTAAG AACAGCAGAT TTGCAGTGGA 660  
ACAGTTCTAA TGCTCAGTTG CTCCTGGACT ACTGCTCTTC CAAGGGGTAT AACATTTCTT 720  
GGGAAGTAGG CAATGAACCT AACAGTTTCC TTAAGAAGGC TGATATTTTC ATCAATGGGT 780  
CGCAGTTAGG AGAAGATTAT ATTCAATTGC ATAACTTCT AAGAAAGTCC ACCTTCAAAA 840  
ATGCAAAACT CTATGGTCCT GATGTTGGTC AGCCTCGAAG AAAGACGGCT AAGATGCTGA 900  
AGAGCTTCCT GAAGGCTGGT GGAGAAGTGA TTGATTCAAT TACATGGCAT CACTACTATT 960  
TGAATGGACG GACTGCTACC AGGGAAGATT TTCTAAACCC TGATGTATTG GACATTTTAA 1020  
TTTCATCTGT GCAAAAAGTT TTCCAGGTGG TTGAGAGCAC CAGGCCTGGC AAGAAGGTCT 1080  
GGTTAGGAGA AACAAGCTCT GCATATGGAG GCGGAGCGCC CTTGCTATCC GACACCTTTG 1140  
CAGCTGGCTT TATGTGGCTG GATAAATTGG GCCTGTCAGC CCGAATGGGA ATAGAAGTGG 1200  
TGATGAGGCA AGTATTCTTT GGAGCAGGAA ACTACCATTI AGTGGATGAA AACTTCGATC 1260  
CTTTACCTGA TTATTGGCTA TCTCTTCTGT TCAAGAAATT GGTGGGCACC AAGGTGTTAA 1320  
TGGCAAGCGT GCAAGGTTCA AAGAGAAGGA AGCTTCGAGT ATACCTTCAT TGCACAAACA 1380  
CTGACAATCC AAGGTATAAA GAAGGAGATT TAACTCTGTA TGCCATAAAC CTCCATAACG 1440  
TCACCAAGTA CTTGCGGTTA CCCTATCCTT TTTCTAACAA GCAAGTGGAT AAATACCTTC 1500  
TAAGACCTTT GGGACCTCAT GGATTACTTT CCAAATCTGT CCAACTCAAT GGTCTAACTC 1560  
TAAAGATGGT GGATGATCAA ACCTTGCCAC CTTTAATGGA AAAACCTCTC CGGCCAGGAA 1620  
GTTCACTGGG CTTGCCAGCT TTCTCATATA GTTTTITITG GATAAGAAAT GCCAAAGTTG 1680  
CTGCTTGCAI CTGAAAATAA AATATACTAG TCCTGACACT G 1721

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

35

- (A) LENGTH: 543
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

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(xi) SEQUENCE DESCRIPTION: SEQ 10 NO:10:

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	Met	Leu	Leu	Arg	Ser	Lys	Pro	Ala	Leu	Pro	Pro	Pro	Leu	Met	Leu	Leu
					5					10				15		
5	Leu	Leu	Gly	Pro	Leu	Gly	Pro	Leu	Ser	Pro	Gly	Ala	Leu	Pro	Arg	Pro
			20						25					30		
	Ala	Gln	Ala	Gln	Asp	Val	Val	Asp	Leu	Asp	Phe	Phe	Thr	Gln	Glu	Pro
			35					40					45			
10	Leu	His	Leu	Val	Ser	Pro	Ser	Phe	Leu	Ser	Val	Thr	Ile	Asp	Ala	Asn
		50					55					60				
	Leu	Ala	Thr	Asp	Pro	Arg	Phe	Leu	Ile	Leu	Leu	Gly	Ser	Pro	Lys	Leu
		65				70					75				80	
	Arg	Thr	Leu	Ala	Arg	Gly	Leu	Ser	Pro	Ala	Tyr	Leu	Arg	Phe	Gly	Gly
					85					90					95	
15	Thr	Lys	Thr	Asp	Phe	Leu	Ile	Phe	Asp	Pro	Lys	Lys	Glu	Ser	Thr	Phe
			100						105					110		
	Glu	Glu	Arg	Ser	Tyr	Trp	Gln	Ser	Gln	Val	Asn	Gln	Asp	Ile	Cys	Lys
			115					120					125			
20	Tyr	Gly	Ser	Ile	Pro	Pro	Asp	Val	Glu	Glu	Lys	Leu	Arg	Leu	Glu	Trp
		130					135					140				
	Pro	Tyr	Gln	Glu	Gln	Leu	Leu	Leu	Arg	Glu	His	Tyr	Gln	Lys	Lys	Phe
		145				150				155					160	
25	Lys	Asn	Ser	Thr	Tyr	Ser	Arg	Ser	Ser	Val	Asp	Val	Leu	Tyr	Thr	Phe
					165					170					175	
	Ala	Asn	Cys	Ser	Gly	Leu	Asp	Leu	Ile	Phe	Gly	Leu	Asn	Ala	Leu	Leu
					180				185					190		
30	Arg	Thr	Ala	Asp	Leu	Gln	Trp	Asn	Ser	Ser	Asn	Ala	Gln	Leu	Leu	Leu
					195			200					205			
	Asp	Tyr	Cys	Ser	Ser	Lys	Gly	Tyr	Asn	Ile	Ser	Trp	Glu	Leu	Gly	Asn
		210					215					220				
35	Glu	Pro	Asn	Ser	Phe	Leu	Lys	Lys	Ala	Asp	Ile	Phe	Ile	Asn	Gly	Ser
		225				230				235					240	
	Gln	Leu	Gly	Glu	Asp	Tyr	Ile	Gln	Leu	His	Lys	Leu	Leu	Arg	Lys	Ser
					245					250					255	
40	Thr	Phe	Lys	Asn	Ala	Lys	Leu	Tyr	Gly	Pro	Asp	Val	Gly	Gln	Pro	Arg
			260						265					270		
	Arg	Lys	Thr	Ala	Lys	Met	Leu	Lys	Ser	Phe	Leu	Lys	Ala	Gly	Gly	Glu
			275					280					285			
45	Val	Ile	Asp	Ser	Val	Thr	Trp	His	His	Tyr	Tyr	Leu	Asn	Gly	Arg	Thr
		290					295						300			
	Ala	Thr	Arg	Glu	Asp	Phe	Leu	Asn	Pro	Asp	Val	Leu	Asp	Ile	Phe	Ile
		305				310				315					320	
	Ser	Ser	Val	Gln	Lys	Val	Phe	Gln	Val	Val	Glu	Ser	Thr	Arg	Pro	Gly
					325					330					335	
50	Lys	Lys	Val	Trp	Leu	Gly	Glu	Thr	Ser	Ser	Ala	Tyr	Gly	Gly	Gly	Ala
					340				345					350		
	Pro	Leu	Leu	Ser	Asp	Thr	Phe	Ala	Ala	Gly	Phe	Met	Trp	Leu	Asp	Lys
					355			360					365			
55	Leu	Gly	Leu	Ser	Ala	Arg	Met	Gly	Ile	Glu	Val	Val	Met	Arg	Gln	Val

	370		375		380	
	Phe Phe Gly Ala Gly Asn Tyr His Leu Val Asp Glu Asn Phe Asp Pro					
5	385		390		395	400
	Leu Pro Asp Tyr Trp Leu Ser Leu Leu Phe Lys Lys Leu Val Gly Thr					
		405		410		415
	Lys Val Leu Met Ala Ser Val Gln Gly Ser Lys Arg Arg Lys Leu Arg					
		420		425		430
10	Val Tyr Leu His Cys Thr Asn Thr Asp Asn Pro Arg Tyr Lys Glu Gly					
		435		440		445
	Asp Leu Thr Leu Tyr Ala Ile Asn Leu His Asn Val Thr Lys Tyr Leu					
		450		455		460
15	Arg Leu Pro Tyr Pro Phe Ser Asn Lys Gln Val Asp Lys Tyr Leu Leu					
		465		470		480
	Arg Pro Leu Gly Pro His Gly Leu Leu Ser Lys Ser Val Gln Leu Asn					
		485		490		495
20	Gly Leu Thr Leu Lys Met Val Asp Asp Gln Thr Leu Pro Pro Leu Met					
		500		505		510
	Glu Lys Pro Leu Arg Pro Gly Ser Ser Leu Gly Leu Pro Ala Phe Ser					
		515		520		525
25	Tyr Ser Phe Phe Val Ile Arg Asn Ala Lys Val Ala Ala Cys Ile					
		530		535		540 543

(2) INFORMATION FOR SEQ ID NO:11:

30 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1718
- (B) TYPE: nucleic acid
- (C) STRAHDEDHESS: double
- 35 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

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**EP 0 998 569 B1**

[illegible]

EP 0 998 569 B1

	TAT GGA TCC ATC CCT CCT GAT GTG GAG GAG AAG TTA CGG TTG GAA TGG	494
	Tyr Gly Ser Ile Pro Pro Asp Val Glu Glu Lys Leu Arg Leu Glu Trp	
	130 135 140	
5	CCC TAC CAG GAG CAA TTG CTA CTC CGA GAA CAC TAC CAG AAA AAG TTC	542
	Pro Tyr Gln Glu Gln Leu Leu Leu Arg Glu His Tyr Gln Lys Lys Phe	
	145 150 155 160	
	AAG AAC AGC ACC TAC TCA AGA AGC TCT GTA GAT GTG CTA TAC ACT TTT	590
	Lys Asn Ser Thr Tyr Ser Arg Ser Ser Val Asp Val Leu Tyr Thr Phe	
	165 170 175	
10	GCA AAC TGC TCA GGA CTG GAC TTG ATC TTT GGC CTA AAT GCG TTA TTA	638
	Ala Asn Cys Ser Gly Leu Asp Leu Ile Phe Gly Leu Asn Ala Leu Leu	
	180 185 190	
	AGA ACA GCA GAT TTG CAG TGG AAC AGT TCT AAT GCT CAG TTG CTC CTG	686
	Arg Thr Ala Asp Leu Gln Trp Asn Ser Ser Asn Ala Gln Leu Leu Leu	
15	195 200 205	
	GAC TAC TGC TCT TCC AAG GGG TAT AAC ATT TCT TGG GAA CTA GGC AAT	734
	Asp Tyr Cys Ser Ser Lys Gly Tyr Asn Ile Ser Trp Glu Leu Gly Asn	
	210 215 220	
20	GAA CCT AAC AGT TTC CTT AAG AAG GCT GAT ATT TTC ATC AAT GGG TCG	782
	Glu Pro Asn Ser Phe Leu Lys Lys Ala Asp Ile Phe Ile Asn Gly Ser	
	225 230 235 240	
	CAG TTA GGA GAA GAT TAT ATT CAA TTG CAT AAA CTT CTA AGA AAG TCC	830
	Gln Leu Gly Glu Asp Tyr Ile Gln Leu His Lys Leu Leu Arg Lys Ser	
	245 250 255	
25	ACC TTC AAA AAT GCA AAA CTC TAT GGT CCT GAT GTT GGT CAG CCT CGA	878
	Thr Phe Lys Asn Ala Lys Leu Tyr Gly Pro Asp Val Gly Gln Pro Arg	
	260 265 270	
	AGA AAG ACG GCT AAG ATG CTG AAG AGC TTC CTG AAG GCT GGT GGA GAA	926
	Arg Lys Thr Ala Lys Met Leu Lys Ser Phe Leu Lys Ala Gly Gly Glu	
30	275 280 285	
	GTG ATT GAT TCA GTT ACA TGG CAT CAC TAC TAT TTG AAT GGA CGG ACT	974
	Val Ile Asp Ser Val Thr Trp His His Tyr Tyr Leu Asn Gly Arg Thr	
	290 295 300	
35	GCT ACC AGG GAA GAT TTT CTA AAC CCT GAT GTA TTG GAC ATT TTT ATT	1019
	Ala Thr Arg Glu Asp Phe Leu Asn Pro Asp Val Leu Asp Ile Phe Ile	
	305 310 315 320	
	TCA TCT GTG CAA AAA GTT TTC CAG GTG GTT GAG AGC ACC AGG CCT GGC	1067
	Ser Ser Val Gln Lys Val Phe Gln Val Val Glu Ser Thr Arg Pro Gly	
	325 330 335	
40	AAG AAG GTC TGG TTA GGA GAA ACA AGC TCT GCA TAT GGA GGC GGA GCG	1115
	Lys Lys Val Trp Leu Gly Glu Thr Ser Ser Ala Tyr Gly Gly Gly Ala	
	340 345 350	
	CCC TTG CTA TCC GAC ACC TTT GCA GCT GGC TTT ATG TGG CTG GAT AAA	1163
	Pro Leu Leu Ser Asp Thr Phe Ala Ala Gly Phe Met Trp Leu Asp Lys	
45	355 360 365	
	TTG GGC CTG TCA GCC CGA ATG GGA ATA GAA GTG GTG ATG AGG CAA GTA	1211
	Leu Gly Leu Ser Ala Arg Met Gly Ile Glu Val Val Met Arg Gln Val	
	370 375 380	
50	TTC TTT GGA GCA GGA AAC TAC CAT TTA GTG GAT GAA AAC TTC GAT CCT	1259
	Phe Phe Gly Ala Gly Asn Tyr His Leu Val Asp Glu Asn Phe Asp Pro	
	385 390 395 400	
	TTA CCT GAT TAT TGG CTA TCT CTT CTG TTC AAG AAA TTG GTG GGC ACC	1307
	Leu Pro Asp Tyr Trp Leu Ser Leu Leu Phe Lys Lys Leu Val Gly Thr	
	405 410 415	
55	AAG GTG TTA ATG GCA AGC GTG CAA GGT TCA AAG AGA AGC AAG CTT CGA	1355
	Lys Val Leu Met Ala Ser Val Gln Gly Ser Lys Arg Arg Lys Leu Arg	

	420	425	430	
5	GTA TAC CTT CAT TGC ACA AAC ACT GAC AAT CCA AGG TAT AAA GAA GGA 1403 Val Tyr Leu His Cys Thr Asn Thr Asp Asn Pro Arg Tyr Lys Glu Gly 435 440 445			
	GAT TTA ACT CTG TAT GCC ATA AAC CTC CAT AAC GTC ACC AAG TAC TTG 1451 Asp Leu Thr Leu Tyr Ala Ile Asn Leu His Asn Val Thr Lys Tyr Leu 450 455 460			
10	CGG TTA CCC TAT CCT TTT TCT AAC AAG CAA GTG GAT AAA TAC CTT CTA 1499 Arg Leu Pro Tyr Pro Phe Ser Asn Lys Gln Val Asp Lys Tyr Leu Leu 465 470 475 480			
	AGA CCT TTG GGA CCT CAT GGA TTA CTT TCC AAA TCT GTC CAA CTC AAT 1547 Arg Pro Leu Gly Pro His Gly Leu Leu Ser Lys Ser Val Gln Leu Asn 485 490 495			
15	GGT CTA ACT CTA AAG ATG GTG GAT GAT CAA ACC TTG CCA CCT TTA ATG 1595 Gly Leu Thr Leu Lys Met Val Asp Asp Gln Thr Leu Pro Pro Leu Met 500 505 510			
20	GAA AAA CCT CTC CGG CCA GGA AGT TCA CTG GGC TTG CCA GCT TTC TCA 1643 Glu Lys Pro Leu Arg Pro Gly Ser Ser Leu Gly Leu Pro Ala Phe Ser 515 520 525			
	TAT AGT TTT TTT GTG ATA AGA AAT GCC AAA GTT GCT GCT TGC ATC TGA 1691 Tyr Ser Phe Phe Val Ile Arg Asn Ala Lys Val Ala Ala Cys Ile 530 535 540 543			
25	AAA TAA AAT ATA CTA GTC CTG ACA CTG 1718			

## (2) INFORMATION FOR SEQ ID NO:12:

## 30 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 824  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: double  
 35 (D) TOPOLOGY: linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:12

40	CTGGCAAGAA GGTCTGGTTG GGAGAGACGA GCTCAGCTTA CGGTGGCGGT GCACCCTTGC 60 TGTCCAACAC CTTTGCAGCT GGCTTTATGT GGCTGGATAA ATTGGGCCTG TCAGCCCAGA 120 TGGGCATAGA AGTCGTGATG AGGCAGGTGT TCTTCGGAGC AGGCAACTAC CACTTAGTGG 180 ATGAAAACCTT TGAGCCTTTA CCTGATTACT GGCTCTCTCT TCTGTTCAAG AAACCTGGTAG 240 GTCCCAGGGT GTTACTGTCA AGAGTGAAAG GCCCAGACAG GAGCAAACCTC CGAGTGTATC 300 TCCACTGCAC TAACGTCTAT CACCCACGAT ATCAGGAAGG AGATCTAACT CTGTATGTCC 360 TGAACCTCCA TAATGTCACC AAGCACTTGA AGGTACCGCC TCCGTTGTTT AGGAAACCAG 420 45 TGGATACGTA CTTTCTGAAG CTTTCGGGGC CGGATGGATT ACTTTCCAAA TCTGTCCAAC 480 TGAACGGTCA AATTCTGAAG ATGGTGGATG AGCAGACCTT GCCAGCTTTG ACAGAAAAAC 540 CTCTCCCCGC AGGAAGTGCA CTAAGCCTGC CTGCCTTTTC CTATGGTTTT TTTGTCATAA 600 GAAATGCCAA AATCGCTGCT TGTATATGAA AATAAAAGGC ATACGGTACC CCTGAGACAA 660 AAGCCGAGGG GGGTGTATT CATAAAACAA AACCTAGTT TAGGAGGCCA CCTCCTTGCC 720 GAGTTCCAGA GCTTCGGGAG GGTGGGGTAC ACTTCAGTAT TACATTCAGT GTGGTGTTC 780 50 CTCTAAGAAG AATACTGCAG GTGGTGACAG TTAATAGCAC TGTG 824
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## (2) INFORMATION FOR SEQ ID NO:13:

## 55 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1899  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: double



(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13

5	GGGAAAGCGA	GCAAGGAAGT	AGGAGAGAGC	CGGGCAGGCG	GGGCGGGGTT	GGATTGGGAG	60
	CAGTGGGAGG	GATGCAGAAG	AGGAGTGGGA	GGGATGGAGG	GCGCAGTGGG	AGGGGTGAGG	120
	AGGCGTAACG	GGGCGGAGGA	AAGGAGAAAA	GGGCGCTGGG	GCTCGGCGGG	AGGAAGTGCT	180
	AGAGCTCTCG	ACTCTCCGCT	GCGCGGCAGC	TGGCGGGGGG	AGCAGCCAGG	TGAGCCCAAG	240
	ATGCTGCTGC	GCTCGAAGCC	TGCGCTGCCG	CCGCCGCTGA	TGCTGCTGCT	CCTGGGGCCG	300
10	CTGGGTCCCC	TCTCCCTGG	CGCCCTGCC	CGACCTGCGC	AAGCACAGGA	CGTCGTGGAC	360
	CTGGACTTCT	TCACCCAGGA	GCCGCTGCAC	CTGGTGAGCC	CCTCGTTCCT	GTCCGTCAAC	420
	ATTGACGCCA	ACCTGGCCAC	GGACCCGCGG	TTCCTCATCC	TCCTGGGTTC	TCCAAAGCTT	480
	CGTACCTTGG	CCAGAGGCTT	GTCTCCTGCG	TACCTGAGGT	TTGGTGGCAC	CAAGACAGAC	540
	TTCCTAATTT	TCGATCCCAA	GAAGGAATCA	ACCTTTGAAG	AGAGAAGTTA	CTGGCAATCT	600
	CAAGTCAACC	AGGATAATTT	CAAATATGGA	TCCATCCCTC	CTGATGTGGA	GGAGAAGTTA	660
15	CGGTGGGAAT	GGCCCTACCA	GGAGCAATTG	CTACTCCGAG	AACACTACCA	GAAAAAGTTC	720
	AAGAACAGCA	CCTACTCAAG	AAGCTCTGTA	GATGTGCTAT	ACACTTTTGC	AAACTGCTCA	780
	GGACTGGACT	TGATCTTTGG	CCTAAATGCG	TTATTAAGAA	CAGCAGATTT	GCAGTGGAAC	840
20	AGTTCTAATG	CTCAGTTGCT	CCTGGACTAC	TGCTCTTCCA	AGGGGTATAA	CATTTCTTGG	900
	GAACTAGGCA	ATGAACCTAA	CAGTTTCCTT	AAGAAGGCTG	ATATTTTCAT	CAATGGGTCTG	960
	CAGTTAGGAG	AAGATTATAT	TCAATTGCAT	AAACTTCTAA	GAAAGTCCAC	CTTCAAAAAT	1020
	GCAAAACTCT	ATGGTCCTGA	TGTTGGTCAG	CCTCGAAGAA	AGACGGCTAA	GATGCTGAAG	1080
	AGCTTCCTGA	AGGCTGGTGG	AGAAGTGATT	GATTCAGTTA	CATGGCATCA	CTACTATTTG	1140
	AATGGACGGA	CTGCTACCAG	GGAAGATTTT	CTAAACCTTG	ATGTATTGGA	CATTTTATT	1200
	TCATCTGTGC	AAAAAGTTTT	CCAGGTGGTT	GAGAGCACCA	GGCCTGGCAA	GAAGGTCTGG	1260
25	TTAGGAGAAA	CAAGCTCTGC	ATATGGAGGC	GGAGCGCCCT	TGCTATCCGA	CACCTTTGCA	1320
	GCTGGCTTTA	TGTGGCTGGA	TAAATTGGGC	CTGTGAGCCC	GAATGGGAAT	AGAAGTGGTG	1380
	ATGAGGCAAG	TATTCTTTGG	AGCAGGAAAC	TACCATTTAG	TGGATGAAAA	CTTCGATCCT	1440
	TTACCTGATT	ATTGGCTATC	TCTTCTGTTT	AAGAAATTGG	TGGGCACCAA	GGTGTTAATG	1500
	GCAAGCGTGC	AAGGTTCAAA	GAGAAGGAAG	CTTCGAGTAT	ACCTTCATTG	CACAAACACT	1560
	GACAATCCAA	GGTATAAAGA	AGGAGATTTA	ACTCTGTATG	CCATAAACCT	CCATAACGTC	1620
30	ACCAAGTACT	TGCGGTACC	CTATCCTTTT	TCTAACAAGC	AAGTGGATAA	ATACCTTCTA	1680
	AGACCTTTGG	GACCTCATGG	ATTACTTTCC	AAATCTGTCC	AACTCAATGG	TCTAACTCTA	1740
	AAGATGGTGG	ATGATCAAAC	CTTGCCACCT	TTAATGGAAA	AACCTCTCCG	GCCAGGAAGT	1800
	TCACTGGGCT	TGCCAGCTTT	CTCATATAGT	TTTTTTGTGA	TAAGAAATGC	CAAAGTTGCT	1860
	GCTTGCACT	GAAAATAAAA	TATACTAGTC	CTGACACTG			1899

35 (2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:

- 40 (A) LENGTH: 592  
(B) TYPE: amino acid  
(C) STRANDEDNESS: singl  
(D) TOPOLOGY: linear

45 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:14

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	Met	Glu	Gly	Ala	Val	Gly	Gly	Val	Arg	Arg	Arg	Asn	Gly	Ala	Glu
					5					10					15
	Glu	Arg	Arg	Lys	Gly	Arg	Trp	Gly	Ser	Ala	Gly	Gly	Ser	Ala	Arg
					20					25					30
5	Ala	Leu	Asp	Ser	Pro	Leu	Arg	Gly	Ser	Trp	Arg	Gly	Glu	Gln	Pro
					35					40					45
	Gly	Glu	Pro	Lys	Met	Leu	Leu	Arg	Ser	Lys	Pro	Ala	Leu	Pro	Pro
					50					55					60
	Pro	Leu	Met	Leu	Leu	Leu	Leu	Gly	Pro	Leu	Gly	Pro	Leu	Ser	Pro
					65					70					75
10	Gly	Ala	Leu	Pro	Arg	Pro	Ala	Gln	Ala	Gln	Asp	Val	Val	Asp	Leu
					80					85					90
	Asp	Phe	Phe	Thr	Gln	Glu	Pro	Leu	His	Leu	Val	Ser	Pro	Ser	Phe
					95					100					105
	Leu	Ser	Val	Thr	Ile	Asp	Ala	Asn	Leu	Ala	Thr	Asp	Pro	Arg	Phe
					110					115					120
15	Leu	Ile	Leu	Leu	Gly	Ser	Pro	Lys	Leu	Arg	Thr	Leu	Ala	Arg	Gly
					125					130					135
	Leu	Ser	Pro	Ala	Tyr	Leu	Arg	Phe	Gly	Gly	Thr	Lys	Thr	Asp	Phe
					140					145					150
	Leu	Ile	Phe	Asp	Pro	Lys	Lys	Glu	Ser	Thr	Phe	Glu	Glu	Arg	Ser
					155					160					165
	Tyr	Trp	Gln	Ser	Gln	Val	Asn	Gln	Asp	Ile	Cys	Lys	Tyr	Gly	Ser
					170					175					180
20	Ile	Pro	Pro	Asp	Val	Glu	Glu	Lys	Leu	Arg	Leu	Glu	Trp	Pro	Tyr
					185					190					195
	Gln	Glu	Gln	Leu	Leu	Leu	Arg	Glu	His	Tyr	Gln	Lys	Lys	Phe	Lys
					200					205					210
	Asn	Ser	Thr	Tyr	Ser	Arg	Ser	Ser	Val	Asp	Val	Leu	Tyr	Thr	Phe
					215					220					225
25	Ala	Asn	Cys	Ser	Gly	Leu	Asp	Leu	Ile	Phe	Gly	Leu	Asn	Ala	Leu
					230					235					240
	Leu	Arg	Thr	Ala	Asp	Leu	Gln	Trp	Asn	Ser	Ser	Asn	Ala	Gln	Leu
					245					250					255
	Leu	Leu	Asp	Tyr	Cys	Ser	Ser	Lys	Gly	Tyr	Asn	Ile	Ser	Trp	Glu
					260					265					270
30	Leu	Gly	Asn	Glu	Pro	Asn	Ser	Phe	Leu	Lys	Lys	Ala	Asp	Ile	Phe
					275					280					285
	Ile	Asn	Gly	Ser	Gln	Leu	Gly	Glu	Asp	Tyr	Ile	Gln	Leu	His	Lys
					290					295					300
	Leu	Leu	Arg	Lys	Ser	Thr	Phe	Lys	Asn	Ala	Lys	Leu	Tyr	Gly	Pro
					305					310					315
35	Asp	Val	Gly	Gln	Pro	Arg	Arg	Lys	Thr	Ala	Lys	Met	Leu	Lys	Ser
					320					325					330
	Phe	Leu	Lys	Ala	Gly	Gly	Glu	Val	Ile	Asp	Ser	Val	Thr	Trp	His
					335					340					345

	His Tyr Tyr Leu	Asn Gly Arg Thr Ala Thr Arg Glu Asp Phe Leu	350	355	360
	Asn Pro Asp Val	Leu Asp Ile Phe Ile Ser Ser Val Gln Lys Val	365	370	375
5	Phe Gln Val Val	Glu Ser Thr Arg Pro Gly Lys Lys Val Trp Leu	380	385	390
	Gly Glu Thr Ser	Ser Ala Tyr Gly Gly Gly Ala Pro Leu Leu Ser	395	400	405
	Asp Thr Phe Ala	Ala Gly Phe Met Trp Leu Asp Lys Leu Gly Leu	410	415	420
10	Ser Ala Arg Met	Gly Ile Glu Val Val Met Arg Gln Val Phe Phe	425	430	435
	Gly Ala Gly Asn	Tyr His Leu Val Asp Glu Asn Phe Asp Pro Leu	440	445	450
	Pro Asp Tyr Trp	Leu Ser Leu Leu Phe Lys Lys Leu Val Gly Thr	455	460	465
15	Lys Val Leu Met	Ala Ser Val Gln Gly Ser Lys Arg Arg Lys Leu	470	475	480
	Arg Val Tyr Leu	His Cys Thr Asn Thr Asp Asn Pro Arg Tyr Lys	485	490	495
	Glu Gly Asp Leu	Thr Leu Tyr Ala Ile Asn Leu His Asn Val Thr	500	505	510
20	Lys Tyr Leu Arg	Leu Pro Tyr Pro Phe Ser Asn Lys Gln Val Asp	515	520	525
	Lys Tyr Leu Leu	Arg Pro Leu Gly Pro His Gly Leu Leu Ser Lys	530	535	540
	Ser Val Gln Leu	Asn Gly Leu Thr Leu Lys Met Val Asp Asp Gln	545	550	555
25	Thr Leu Pro Pro	Leu Met Glu Lys Pro Leu Arg Pro Gly Ser Ser	560	565	570
	Leu Gly Leu Pro	Ala Phe Ser Tyr Ser Phe Phe Val Ile Arg Asn	575	580	585
	Ala Lys Val Ala	Ala Cys Ile	590	592	

30 (2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:

- |    |                          |
|----|--------------------------|
| 35 | (A) LENGTH: 1899         |
|    | (B) TYPE: nucleic acid   |
|    | (C) STRANDEDNESS: double |
|    | (D) TOPOLOGY: linear     |

40 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:15

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																	GGG	3
	AAA	GCG	AGC	AAG	GAA	GTA	GGA	GAG	AGC	CGG	GCA	GGC	GGG	GCG	GGG			48
	TTG	GAT	TGG	GAG	CAG	TGG	GAG	GGA	TGC	AGA	AGA	GGA	GTG	GGA	GGG			93
	ATG	GAG	GGC	GCA	GTG	GGA	GGG	GTG	AGG	AGG	CGT	AAC	GGG	GCG	GAG			138
5	Met	Glu	Gly	Ala	Val	Gly	Gly	Val	Arg	Arg	Arg	Asn	Gly	Ala	Glu			
					5					10					15			
	GAA	AGG	AGA	AAA	GGG	CGC	TGG	GGC	TCG	GCG	GGA	GGA	AGT	GCT	AGA			183
	Glu	Arg	Arg	Lys	Gly	Arg	Trp	Gly	Ser	Ala	Gly	Gly	Ser	Ala	Arg			
					20					25					30			
10																		
	GCT	CTC	GAC	TCT	CCG	CTG	CGC	GGC	AGC	TGG	CGG	GGG	GAG	CAG	CCA			228
	Ala	Leu	Asp	Ser	Pro	Leu	Arg	Gly	Ser	Trp	Arg	Gly	Glu	Gln	Pro			
					35					40					45			
	GGT	GAG	CCC	AAG	ATG	CTG	CTG	CGC	TCG	AAG	CCT	GCG	CTG	CCG	CCG			273
15	Gly	Glu	Pro	Lys	Met	Leu	Leu	Arg	Ser	Lys	Pro	Ala	Leu	Pro	Pro			
					50					55					60			
	CCG	CTG	ATG	CTG	CTG	CTC	CTG	GGG	CCG	CTG	GGT	CCC	CTC	TCC	CCT			318
	Pro	Leu	Met	Leu	Leu	Leu	Leu	Gly	Pro	Leu	Gly	Pro	Leu	Ser	Pro			
					65					70					75			
	GGC	GCC	CTG	CCC	CGA	CCT	GCG	CAA	GCA	CAG	GAC	GTc	GTG	GAC	CTG			363
20	Gly	Ala	Leu	Pro	Arg	Pro	Ala	Gln	Ala	Gln	Asp	Val	Val	Asp	Leu			
					80					85					90			
	GAC	TTC	TTC	ACC	CAG	GAG	CCG	CTG	CAC	CTG	GTG	AGC	CCC	TCG	TTC			408
	Asp	Phe	Phe	Thr	Gln	Glu	Pro	Leu	His	Leu	Val	Ser	Pro	Ser	Phe			
					95					100					105			
25																		
	CTG	TCC	GTC	ACC	ATT	GAC	GCC	AAC	CTG	GCC	ACG	GAC	CCG	CGG	TTC			453

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	Leu	Ser	Val	Thr	Ile	Asp	Ala	Asn	Leu	Ala	Thr	Asp	Pro	Arg	Phe	
					110					115					120	
5	CTC	ATC	CTC	CTG	GGT	TCT	CCA	AAG	CTT	CGT	ACC	TTG	GCC	AGA	GGC	498
	Leu	Ile	Leu	Leu	Gly	Ser	Pro	Lys	Leu	Arg	Thr	Leu	Ala	Arg	Gly	
					125					130					135	
	TTG	TCT	CCT	GCG	TAC	CTG	AGG	TTT	GGT	GGC	ACC	AAG	ACA	GAC	TTC	543
	Leu	Ser	Pro	Ala	Tyr	Leu	Arg	Phe	Gly	Gly	Thr	Lys	Thr	Asp	Phe	
					140					145					150	
10	CTA	ATT	TTC	GAT	CCC	AAG	AAG	GAA	TCA	ACC	TTT	GAA	GAG	AGA	AGT	588
	Leu	Ile	Phe	Asp	Pro	Lys	Lys	Glu	Ser	Thr	Phe	Glu	Glu	Arg	Ser	
					155					160					165	
	TAC	TGG	CAA	TCT	CAA	GTC	AAC	CAG	GAT	ATT	TGC	AAA	TAT	GGA	TCC	633
15	Tyr	Trp	Gln	Ser	Gln	Val	Asn	Gln	Asp	Ile	Cys	Lys	Tyr	Gly	Ser	
					170					175					180	
	ATC	CCT	CCT	GAT	GTG	GAG	GAG	AAG	TTA	CGG	TTG	GAA	TGG	CCC	TAC	678
	Ile	Pro	Pro	Asp	Val	Glu	Glu	Lys	Leu	Arg	Leu	Glu	Trp	Pro	Tyr	
					185					190					195	
20	CAG	GAG	CAA	TTG	CTA	CTC	CGA	GAA	CAC	TAC	CAG	AAA	AAG	TTC	AAG	723
	Gln	Glu	Gln	Leu	Leu	Leu	Arg	Glu	His	Tyr	Gln	Lys	Lys	Phe	Lys	
					200					205					210	
	AAC	AGC	ACC	TAC	TCA	AGA	AGC	TCT	GTA	GAT	GTG	CTA	TAC	ACT	TTT	768
	Asn	Ser	Thr	Tyr	Ser	Arg	Ser	Ser	Val	Asp	Val	Leu	Tyr	Thr	Phe	
					215					220					225	
25	GCA	AAC	TGC	TCA	GGA	CTG	GAC	TTG	ATC	TTT	GGC	CTA	AAT	GCG	TTA	813
	Ala	Asn	Cys	Ser	Gly	Leu	Asp	Leu	Ile	Phe	Gly	Leu	Asn	Ala	Leu	
					230					235					240	
	TTA	AGA	ACA	GCA	GAT	TTG	CAG	TGG	AAC	AGT	TCT	AAT	GCT	CAG	TTG	858
30	Leu	Arg	Thr	Ala	Asp	Leu	Gln	Trp	Asn	Ser	Ser	Asn	Ala	Gln	Leu	
					245					250					255	
	CTC	CTG	GAC	TAC	TGC	TCT	TCC	AAG	GGG	TAT	AAC	ATT	TCT	TGG	GAA	903
	Leu	Leu	Asp	Tyr	Cys	Ser	Ser	Lys	Gly	Tyr	Asn	Ile	Ser	Trp	Glu	
					260					265					270	
35	CTA	GGC	AAT	GAA	CCT	AAC	AGT	TTC	CTT	AAG	AAG	GCT	GAT	ATT	TTC	948
	Ile	Gly	Asn	Glu	Pro	Asn	Ser	Phe	Leu	Lys	Lys	Ala	Asp	Ile	Phe	
					275					280					285	
	ATC	AAT	GGG	TCG	CAG	TTA	GGA	GAA	GAT	TAT	ATT	CAA	TTG	CAT	AAA	993
	Ile	Asn	Gly	Ser	Gln	Leu	Gly	Glu	Asp	Tyr	Ile	Gln	Leu	His	Lys	
					290					295					300	
40	CTT	CTA	AGA	AAG	TCC	ACC	TTC	AAA	AAT	GCA	AAA	CTC	TAT	GGT	CCT	1038
	Leu	Leu	Arg	Lys	Ser	Thr	Phe	Lys	Asn	Ala	Lys	Leu	Tyr	Gly	Pro	
					305					310					315	
	GAT	GTT	GGT	CAG	CCT	CGA	AGA	AAG	ACG	GCT	AAG	ATG	CTG	AAG	AGC	1083
45	Asp	Val	Gly	Gln	Pro	Arg	Arg	Lys	Thr	Ala	Lys	Met	Leu	Lys	Ser	
					320					325					330	
	TTC	CTG	AAG	GCT	GGT	GGA	GAA	GTG	ATT	GAT	TCA	GTT	ACA	TGG	CAT	1128
	Phe	Leu	Lys	Ala	Gly	Gly	Glu	Val	Ile	Asp	Ser	Val	Thr	Trp	His	
					335					340					345	
50	CAC	TAC	TAT	TTG	AAT	GGA	CGG	ACT	GCT	ACC	AGG	GAA	GAT	TTT	CTA	1173
	His	Tyr	Tyr	Leu	Asn	Gly	Arg	Thr	Ala	Thr	Arg	Glu	Asp	Phe	Leu	
					350					355					360	
	AAC	CCT	GAT	GTA	TTG	GAC	ATT	TTT	ATT	TCA	TCT	GTG	CAA	AAA	GTT	1218
	Asn	Pro	Asp	Val	Leu	Asp	Ile	Phe	Ile	Ser	Ser	Val	Gln	Lys	Val	
					365					370					375	
55	TTC	CAG	GTG	GTT	GAG	AGC	ACC	AGG	CCT	GGC	AAG	AAG	GTG	TGG	TTA	1263
	Phe	Gln	Val	Val	Glu	Ser	Thr	Arg	Pro	Gly	Lys	Lys	Val	Trp	Leu	
					380					385					390	



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	GGA GAA ACA AGC TCT GCA TAT GGA GGC GGA GCG CCC TTG CTA TCC	1308
	Gly Glu Thr Ser Ser Ala Tyr Gly Gly Gly Ala Pro Leu Leu Ser	
	395 400 405	
5	GAC ACC TTT GCA GCT GGC TTT ATG TGG CTG GAT AAA TTG GGC CTG	1353
	Asp Thr Phe Ala Ala Gly Phe Met Trp Leu Asp Lys Leu Gly Leu	
	410 415 420	
10	TCA GCC CGA ATG GGA ATA gAA GTG GTG ATG AGG CAA GTA TTC TTT	1398
	Ser Ala Arg Met Gly Ile Glu Val Val Met Arg Gln Val Phe Phe	
	425 430 435	
	GGA GCA GGA AAC TAC CAT TTA GTG GAT GAA AAC TTC GAT CCT TTA	1443
	Gly Ala Gly Asn Tyr His Leu Val Asp Glu Asn Phe Asp Pro Leu	
	440 445 450	
15	CCT GAT TAT TGG CTA TCT CTT CTG TTC AAG AAA TTG GTG GGC ACC	1488
	Pro Asp Tyr Trp Leu Ser Leu Leu Phe Lys Lys Leu Val Gly Thr	
	455 460 465	
	AAG GTG TTA ATG GCA AGC GTG CAA GGT TCA AAG AGA AGG AAG CTT	1533
	Lys Val Leu Met Ala Ser Val Gln Gly Ser Lys Arg Arg Lys Leu	
	470 475 480	
20	CGA GTA TAC CTT CAT TGC ACA AAC ACT GAC AAT CCA AGG TAT AAA	1578
	Arg Val Tyr Leu His Cys Thr Asn Thr Asp Asn Pro Arg Tyr Lys	
	485 490 495	
25	GAA GGA GAT TTA ACT CTG TAT GCC ATA AAC CTC CAT AAC GTC ACC	1623
	Glu Gly Asp Leu Thr Leu Tyr Ala Ile Asn Leu His Asn Val Thr	
	500 505 510	
	AAG TAC TTG CGG TTA CCC TAT CCT TTT TCT AAC AAG CAA GTG GAT	1668
	Lys Tyr Leu Arg Leu Pro Tyr Pro Phe Ser Asn Lys Gln Val Asp	
	515 520 525	
30	AAA TAC CTT CTA AGA CCT TTG GGA CCT CAT GGA TTA CTT TCC AAA	1713
	Lys Tyr Leu Leu Arg Pro Leu Gly Pro His Gly Leu Leu Ser Lys	
	530 535 540	
	TCT GTC CAA CTC AAT GGT CTA ACT CTA AAG ATG GTG GAT GAT CAA	1758
	Ser Val Gln Leu Asn Gly Leu Thr Leu Lys Met Val Asp Asp Gln	
	545 550 555	
35	ACC TTG CCA CCT TTA ATG GAA AAA CCT CTC CGG CCA GGA AGT TCA	1803
	Thr Leu Pro Pro Leu Met Glu Lys Pro Leu Arg Pro Gly Ser Ser	
	560 565 570	
40	CTG GGC TTG CCA GCT TTC TCA TAT AGT TTT TTT GTG ATA AGA AAT	1848
	Leu Gly Leu Pro Ala Phe Ser Tyr Ser Phe Phe Val Ile Arg Asn	
	575 580 585	
	GCC AAA GTT GCT GCT TGC ATC TGA AAA TAA AAT ATA CTA GTC CTG	1893
	Ala Lys Val Ala Ala Cys Ile	
	590 592	
45	ACA CTG	1899

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 594
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16

ATTACTATAG GGCACGCGTG GTCGACGGCC CGGGCTGGTA TTGTCTTAAT GAGAAGTTGA 60  
TAAAGAATTI TGGGTGGTIG ATCTCTTTC AGCTGCAGTT TAGCGTATGC TGAGGCCAGA 120  
TTTTTTCAGG CAAAAGTAAA ATACCTGAGA AACTGCCTGG CCAGAGGACA ATCAGATTTT 180  
GGCTGGCTCA AGTGACAAGC AAGTGTTTAT AAGCTAGATG GGAGAGGAAG GGATGAATAC 240  
TCCATTGGAG GCTTTACTCG AGGGTCAGAG GGATACCCGG CGCCATCAGA ATGGGATCTG 300  
GGAGTCGGAA ACGCTGGGTI CCCACGAGAG CGCGCAGAAC ACGTGCGTCA GGAAGCCTGG 360  
TCCGGGATGC CCAGCGCTGC TCCCCGGGCG CTCCTCCCCG GCGGCTCCTC CCCAGGECTC 420  
CCGGGCGCTI GGATCCCGGC CATCTCCGCA CCCTTCAAGT GGGTGTGGGT GATTTCGTAA 480  
GTGAACGTGA CCGCCACCGG GGGGAAAGCG AGCAAGGAAG TAGGAGAGAG CCGGGCAGGC 540  
GGGCGGGGT TGGATTGGGA GCAGTGGGAG GGATGCAGAA GAGGAGTGGG AGGG 594

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(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:

15

- (A) LENGTH: 21
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17

CCCCAGGAGC AGCAGCATCA G 21

25

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:

30

- (A) LENGTH: 21
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18

AGGCTTCGAG CGCAGCAGCA T 21

40

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:

45

- (A) LENGTH: 22
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19

GTAATACGAC TCACTATAGG GC 22

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(2) INFORMATION FOR SEQ 10 NO:20:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 19
- (B) TYPE: nucleic acid

(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20

5

ACTATAGGGC ACGCGTGGT 19

(2) INFORMATION FOR SEQ ID NO:21: .

10

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

15

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21

20

CTTGGGCTCA CCTGGCTGCT C 21

(2) INFORMATION FOR SEQ ID NO:22:

25

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 23  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

30

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22

35

AGCTCTGTAG ATGTGCTATA CAC 23

(2) INFORMATION FOR SEQ ID NO:23:

40

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 22  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

45

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23

50

GCATCTTAGC CGTCTTTCTT CG 22

## Claims

1. An isolated polynucleotide fragment comprising a polynucleotide sequence encoding a polypeptide having heparanase catalytic activity, wherein said polypeptide shares at least 70% homology with SEQ ID NOs: 10 or 14 or a functional fragment thereof having heparanase catalytic activity.
2. The polynucleotide fragment of claim 1, wherein said polynucleotide sequence includes nucleotides 63 - 1691 of

SEQ ID NO: 9 or nucleotides 139 - 1869 of SEQ ID NO: 13.

3. The polynucleotide fragment according to claim 1 or 2, wherein said polynucleotide sequence includes nucleotides 63 - 721 of SEQ ID NO- 9.
4. The polynucleotide, fragment of claim 1, wherein said polynucleotide is as set forth in SEQ ID NO: 9 or 13.
5. The polynucleotide fragment of claim 1, wherein said polypeptide includes an amino acid sequence as set forth in SEQ ID NOs: 10 or 14.
6. A polynucleotide fragment comprising a polynucleotide sequence at least 70% homologous with SEQ ID NOs: 9 or 13, said polynucleotide sequence encoding a polypeptide having heparanase catalytic activity.
7. The polynucleotide fragment of any preceding claim, wherein said polynucleotide sequence is selected from the group consisting of double stranded DNA, single stranded DNA and RNA.
8. A vector comprising a polynucleotide fragment according to any preceding claim.
9. A host cell comprising an exogenous polynucleotide fragment according to any of claims 1 to 7.
10. A recombinant protein, which is a polypeptide of 543 amino acids as set forth in SEQ ID NO: 10 with a calculated molecular weight of 61,192 daltons or a functional part thereof
11. A polypeptide of 592 amino acids as set forth in SEQ ID NO: 14 with a calculated molecular weight of 66,407 daltons or a functional part thereof.
12. A pharmaceutical composition comprising, as an active ingredient, the isolated protein/polypeptide according to claim 10 or 11 and a pharmaceutically acceptable carrier.
13. A medical device containing, as an active ingredient, an isolated protein/polypeptide according to claim 10 or 11.
14. A heparanase overexpression system comprising a cell overexpressing heparanase sharing at least 70% homology with SEQ ID Nos: 10 or 14 and encoded by a polynucleotide fragment as claimed in any one of claims 1 to 7.
15. A method of identifying a chromosome region harbouring a heparanase gene in a chromosome spread comprising the steps of:
  - (a) hybridizing the chromosome spread with a tagged polynucleotide probe according to claims 6 or 7,
  - (b) washing the chromosome spread, thereby removing excess of non-hybridized probe; and
  - (c) searching for signals associated with said hybridized tagged probe, wherein detected signals being indicative of a chromosome region harbouring a heparanase gene.

#### Patentansprüche

1. Isoliertes Polynucleotidfragment, umfassend eine Polynucleotidsequenz, die ein Polypeptid codiert, das Heparanase-katalytische Aktivität besitzt, wobei das Polypeptid mindestens 70% Homologie mit SEQ ID NR:10 oder 14 oder einem funktionalen Fragment davon mit Heparanase-katalytischer Aktivität aufweist.
2. Polynucleotidfragment nach Anspruch 1, wobei die Polynucleotidsequenz Nucleotide 63-1691 von SEQ ID NR:9 oder Nucleotide 139-1869 von SEQ ID NR:13 umfasst.
3. Polynucleotidfragment nach Anspruch 1 oder 2, wobei die Polynucleotidsequenz Nucleotide 63-721 von SEQ ID NR:9 umfasst.
4. Polynucleotidfragment nach Anspruch 1, wobei das Polynucleotid wie in SEQ ID NR:9 oder 13 dargestellt ist.
5. Polynucleotidfragment nach Anspruch 1, wobei das Polypeptid eine wie in SEQ ID NR:10 oder 14 dargestellte



Aminosäuresequenz umfasst.

6. Polynucleotidfragment, umfassend eine Polynucleotidsequenz mit mindestens 70% Homologie mit SEQ ID NR:9 oder 13, wobei die Polynucleotidsequenz ein Polypeptid mit Heparanase-katalytischer Aktivität codiert.
7. Polynucleotidfragment nach einem der vorangegangenen Ansprüche, wobei die Polynucleotidsequenz ausgewählt ist aus einer Gruppe bestehend aus doppelsträngiger DNA, einzelsträngiger DNA und RNA.
8. Vektor, umfassend ein Polynucleotidfragment nach einem der vorangegangenen Ansprüche.
9. Wirtszelle, umfassend ein exogenes Polynucleotidfragment nach einem der Ansprüche 1 bis 7.
10. Rekombinantes Protein, welches ein Polypeptid mit 543 Aminosäuren ist, wie dargestellt in SEQ ID NR:10, mit einem berechneten Molekulargewicht von 61.192 Dalton, oder ein funktionelles Fragment davon.
11. Polypeptid mit 592 Aminosäuren, wie dargestellt in SEQ ID NR:14, mit einem berechneten Molekulargewicht von 66.407 Dalton, oder ein funktionelles Fragment davon.
12. Arzneimittel, umfassend, als einen aktiven Bestandteil, das isolierte Protein/Polypeptid nach Anspruch 10 oder 11 und einen pharmazeutisch verträglichen Träger.
13. Medizinprodukt, enthaltend, als einen aktiven Bestandteil, ein isoliertes Protein/Polypeptid nach Anspruch 10 oder 11.
14. Heparanase-Überexpressionssystem, umfassend eine Zelle, welche eine Heparanase überexprimiert, die mindestens 70% Homologie mit SEQ ID NR:10 oder 14 aufweist und durch ein Polynucleotidfragment gemäß einem der Ansprüche 1 bis 7 codiert wird.
15. Verfahren zur Identifizierung einer Chromosomenregion, die ein Heparanasegen enthält, in einer Chromosomenansammlung, umfassend die Schritte:
  - (a) Hybridisieren der Chromosomenansammlung mit einer markierten Polynucleotidsonde nach einem der Ansprüche 6 oder 7;
  - (b) Waschen der Chromosomenansammlung, wobei ein Überschuss von nicht hybridisierter Sonde entfernt wird; und
  - (c) Suchen nach Signalen, die mit der hybridisierten markierten Sonde assoziiert sind, wobei der Nachweis von Signalen auf eine Chromosomenregion hinweist, die ein Heparanasegen enthält.

#### Revendications

1. Fragment polynucléotidique isolé comprenant une séquence polynucléotidique codant un polypeptide ayant une activité catalytique héparanase, dans lequel ledit polypeptide possède au moins 70% d'homologie avec SEQ ID NO : 10 ou 14 ou un fragment fonctionnel de celles-ci ayant une activité catalytique héparanase.
2. Fragment polynucléotidique selon la revendication 1, dans lequel la séquence polynucléotidique inclut les nucléotides 63 - 1691 de SEQ ID NO : 9 ou les nucléotides 139 -1869 de SEQ ID NO 13.
3. Fragment polynucléotidique selon la revendication 1 ou 2, dans lequel la séquence polynucléotidique inclut les nucléotides 63 - 721 de SEQ ID NO : 9.
4. Fragment polynucléotidique selon la revendication 1, dans lequel ledit polynucléotide est tel que représenté dans SEQ ID NO : 9 ou 13.
5. Fragment polynucléotidique selon la revendication 1, dans lequel ledit polypeptide inclut une séquence d'acides aminés telle que représentée dans SEQ ID NO : 10 ou 14.
6. Fragment polynucléotidique comprenant une séquence polynucléotidique au moins 70% homologue à SEQ ID

NOs : 9 ou 13, ladite séquence polynucléotidique codant un polypeptide ayant une activité catalytique héparanase.

7. Fragment polynucléotidique selon l'une quelconque des revendications précédentes, dans lequel ladite séquence polynucléotidique est choisie dans le groupe constitué d'ADN double-brin, d'ADN simple-brin et d'ARN.

8. Vecteur comprenant un fragment polynucléotidique selon l'une quelconque des revendications précédentes.

9. Cellule hôte comprenant un fragment polynucléotidique exogène selon l'une des revendications 1 à 7.

10. Protéine recombinante, qui est un polypeptide de 543 acides aminés tel que représenté dans SEQ ID NO : 10 ayant un poids moléculaire calculé de 61 192 daltons, ou une partie fonctionnelle de celui-ci.

11. Polypeptide de 592 acides aminés tel que représenté dans SEQ ID NO : 14 ayant un poids moléculaire calculé de 66 407 daltons, ou une partie fonctionnelle de celui-ci.

12. Composition pharmaceutique comprenant, en tant qu'ingrédient actif, la(le) protéine/polypeptide isolé(e) selon la revendication 10 ou 11 et un support acceptable sur le plan pharmaceutique.

13. Dispositif médical contenant, en tant qu'ingrédient actif, un(e) protéine/polypeptide isolé(e) selon la revendication 10 ou 11.

14. Système pour la sur-expression d'une héparanase, comprenant une cellule sur-exprimant une héparanase ayant au moins 70% d'homologie avec SEQ ID NO : 10 ou 14 et codée par un fragment polynucléotidique tel que revendiqué dans l'une des revendications 1 à 7.

15. Méthode pour identifier une région chromosomique portant un gène héparanase à partir d'un étalement de chromosomes, comprenant les étapes consistant à :

(a) hybrider l'étalement de chromosomes avec une sonde polynucléotidique étiquetée selon les revendications 6 ou 7,

(b) laver l'étalement de chromosomes, éliminant ainsi l'excès de sonde non-hybridée ; et

(c) rechercher des signaux associés à ladite sonde étiquetée, les signaux détectés étant indicatifs d'une région chromosomique portant un gène héparanase.

[illegible]

FIG. 2

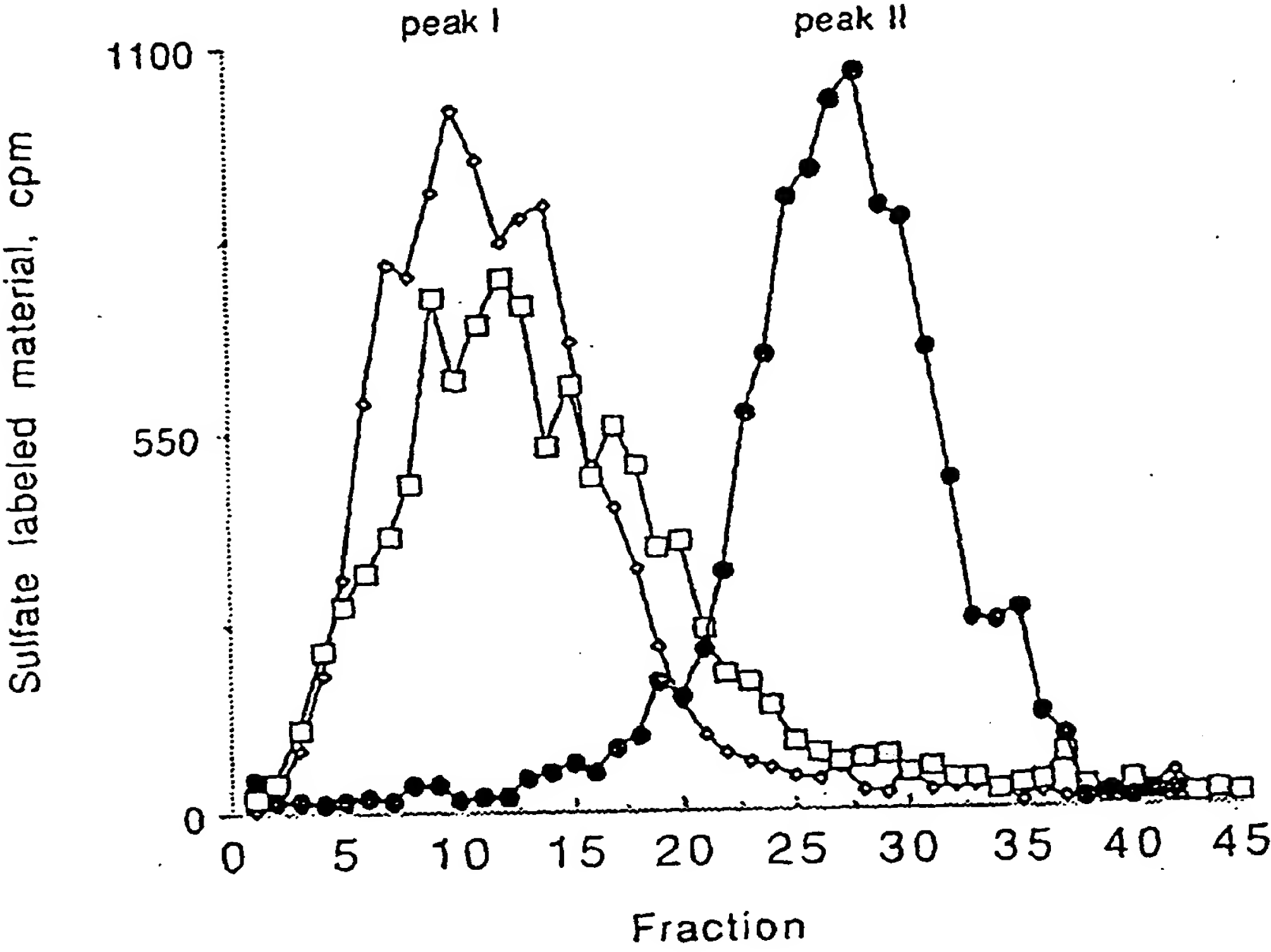




FIG. 3A

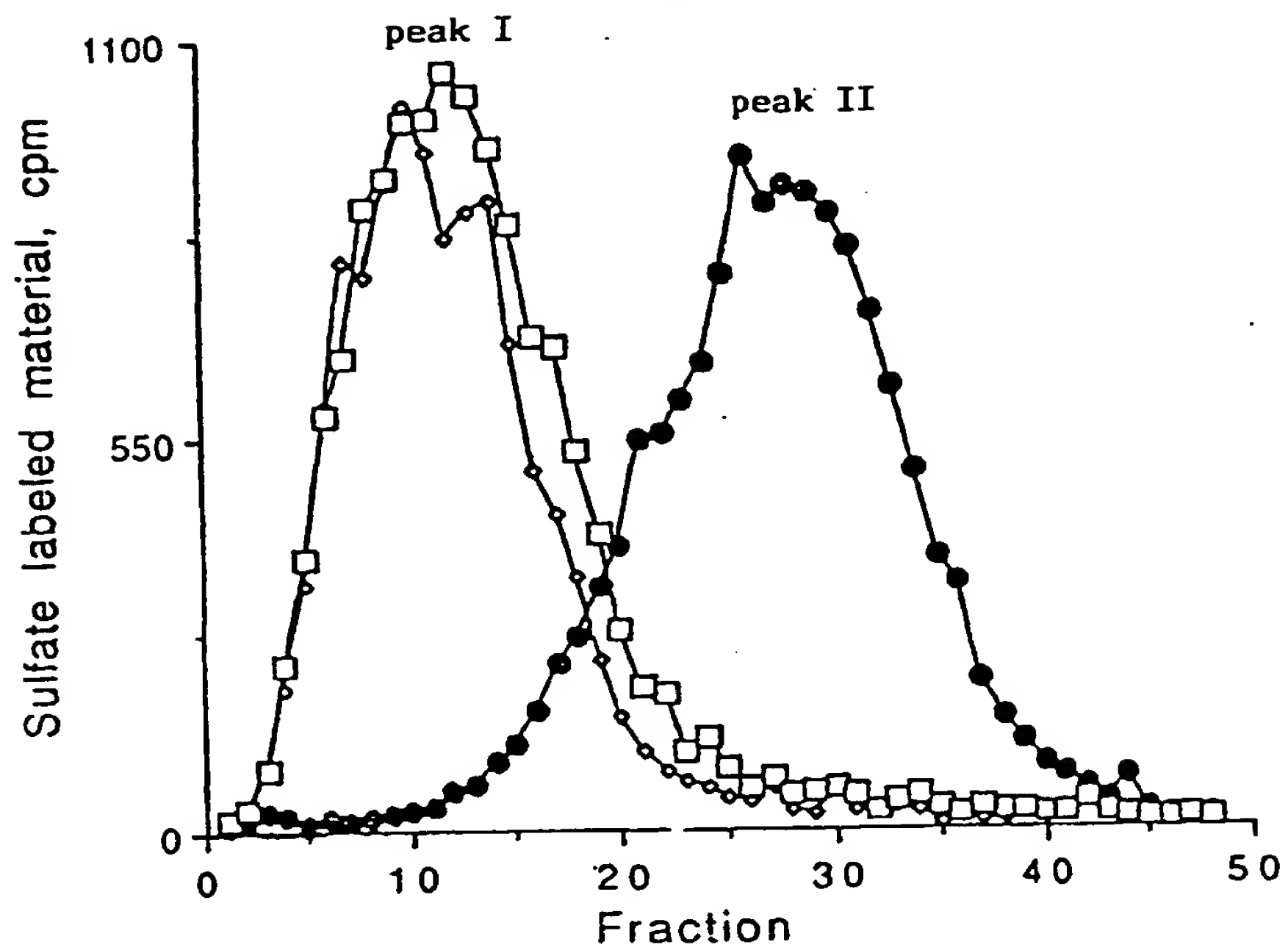


FIG. 3B

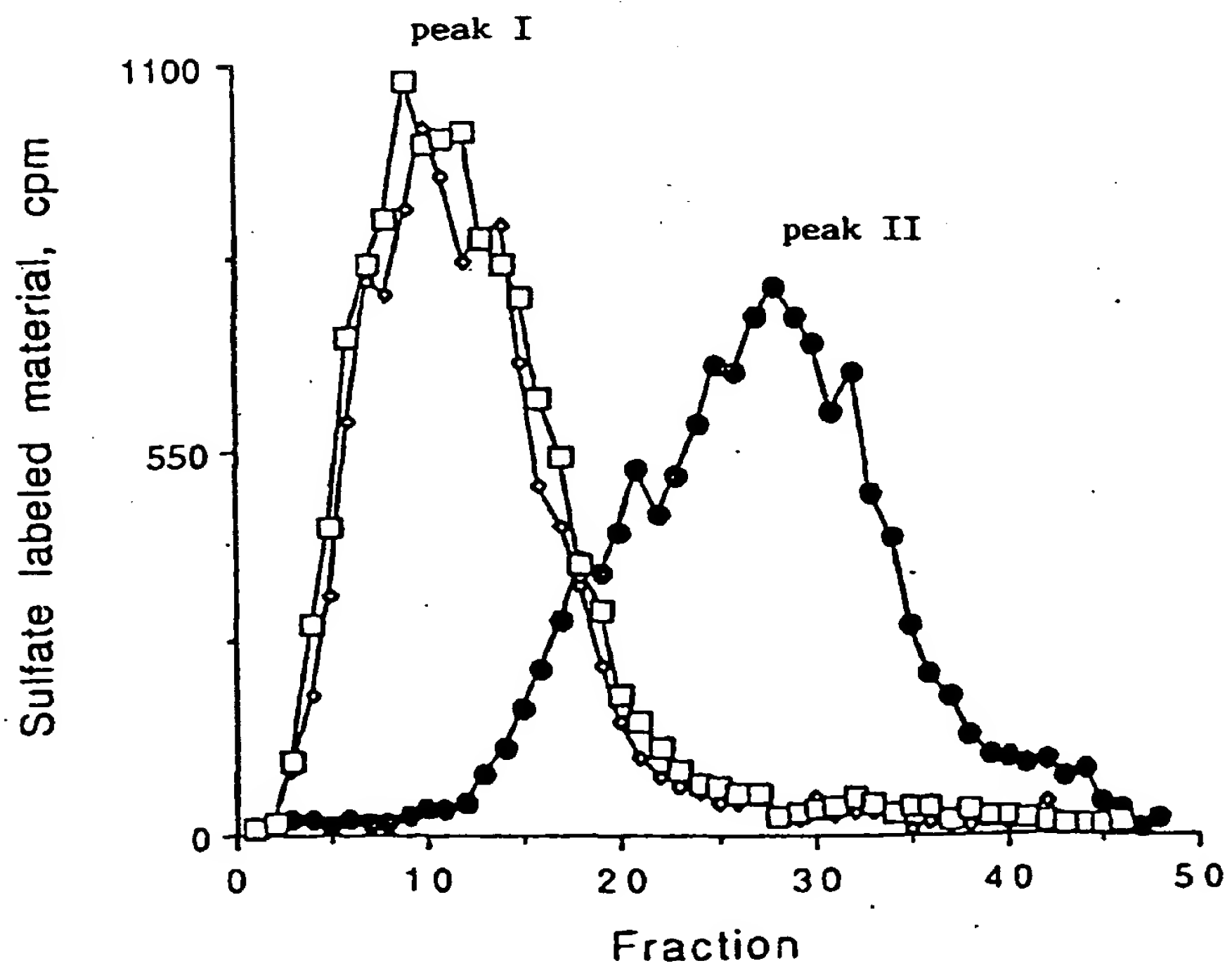


FIG. 4

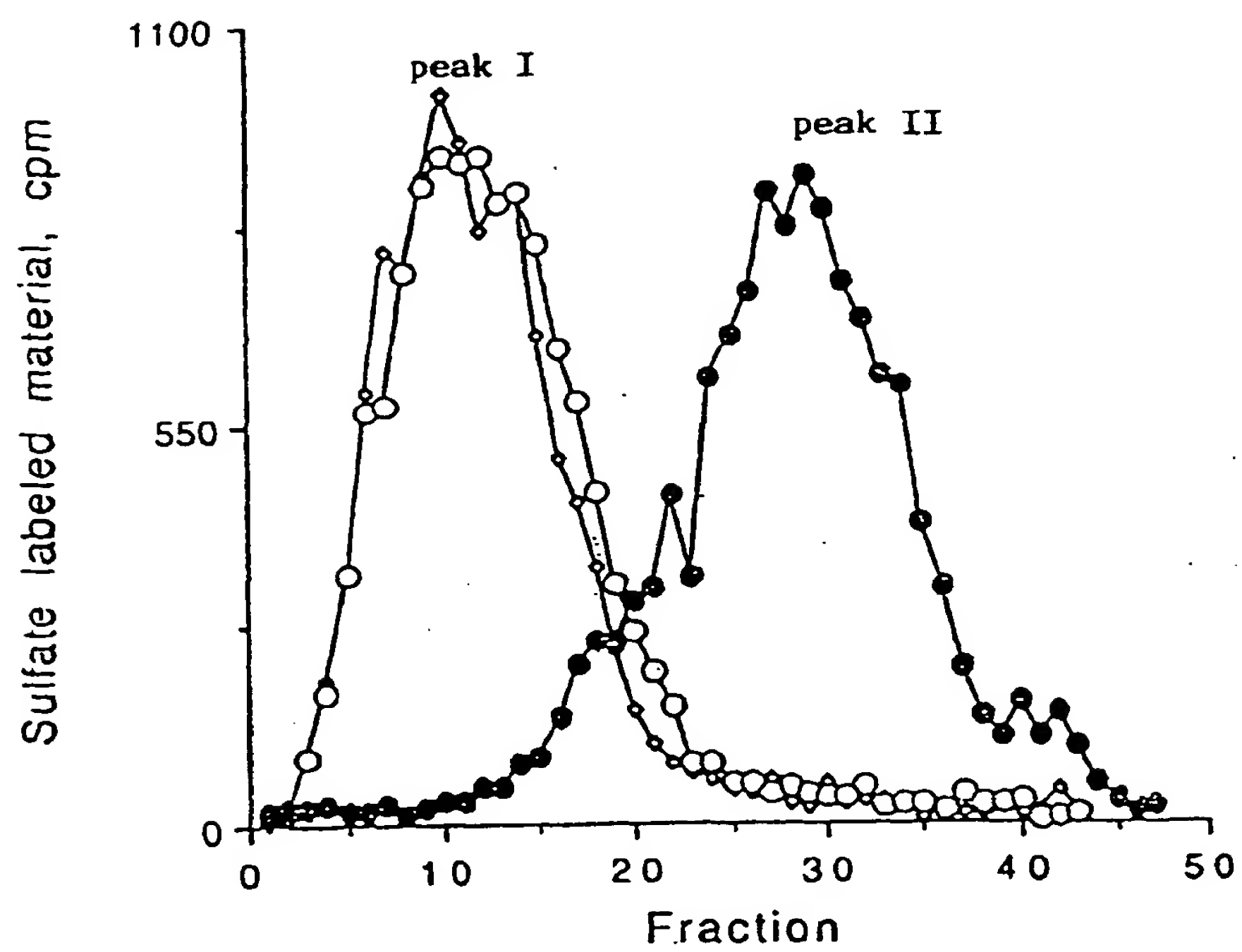


FIG. 5A

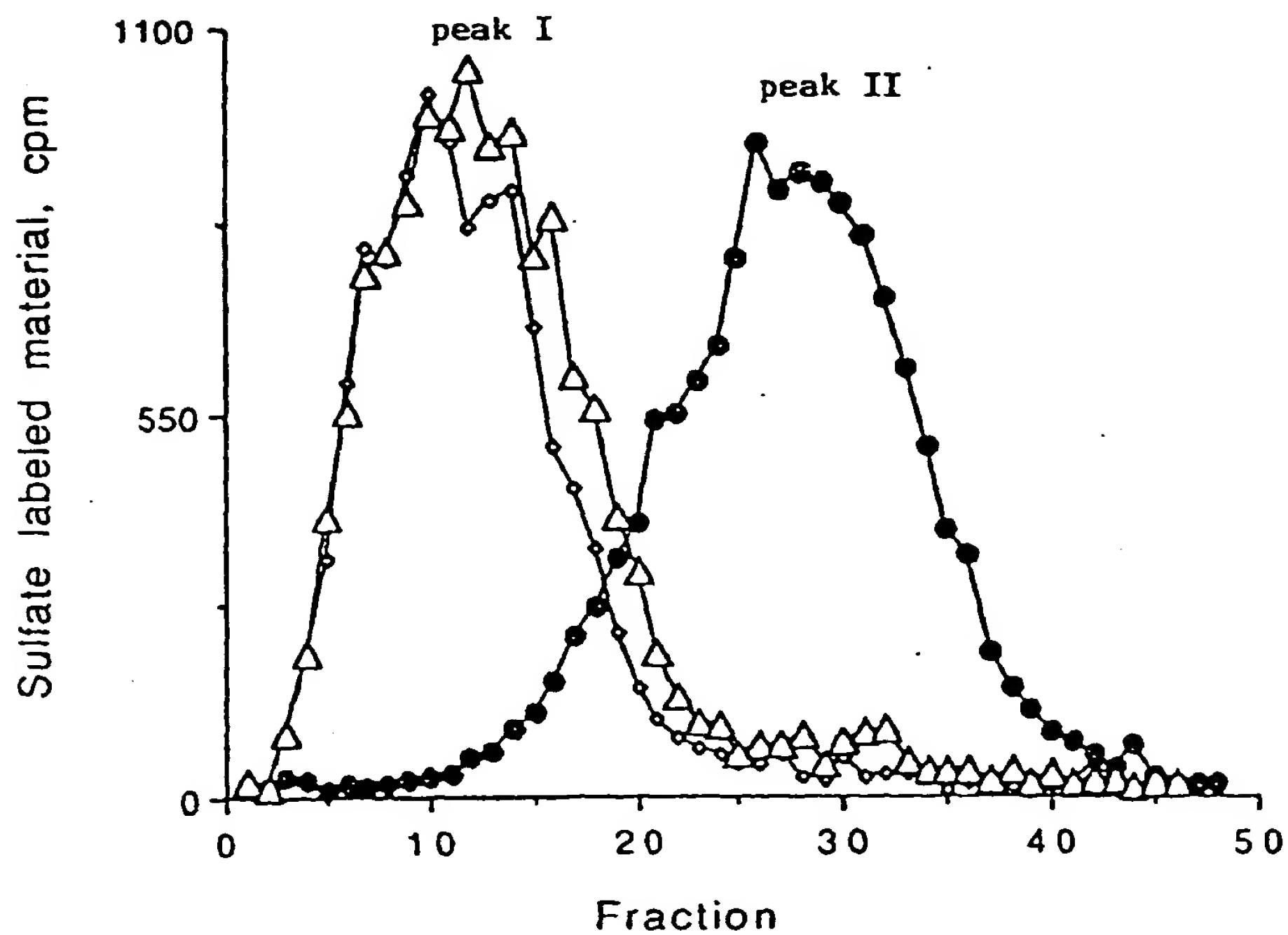


FIG. 5B

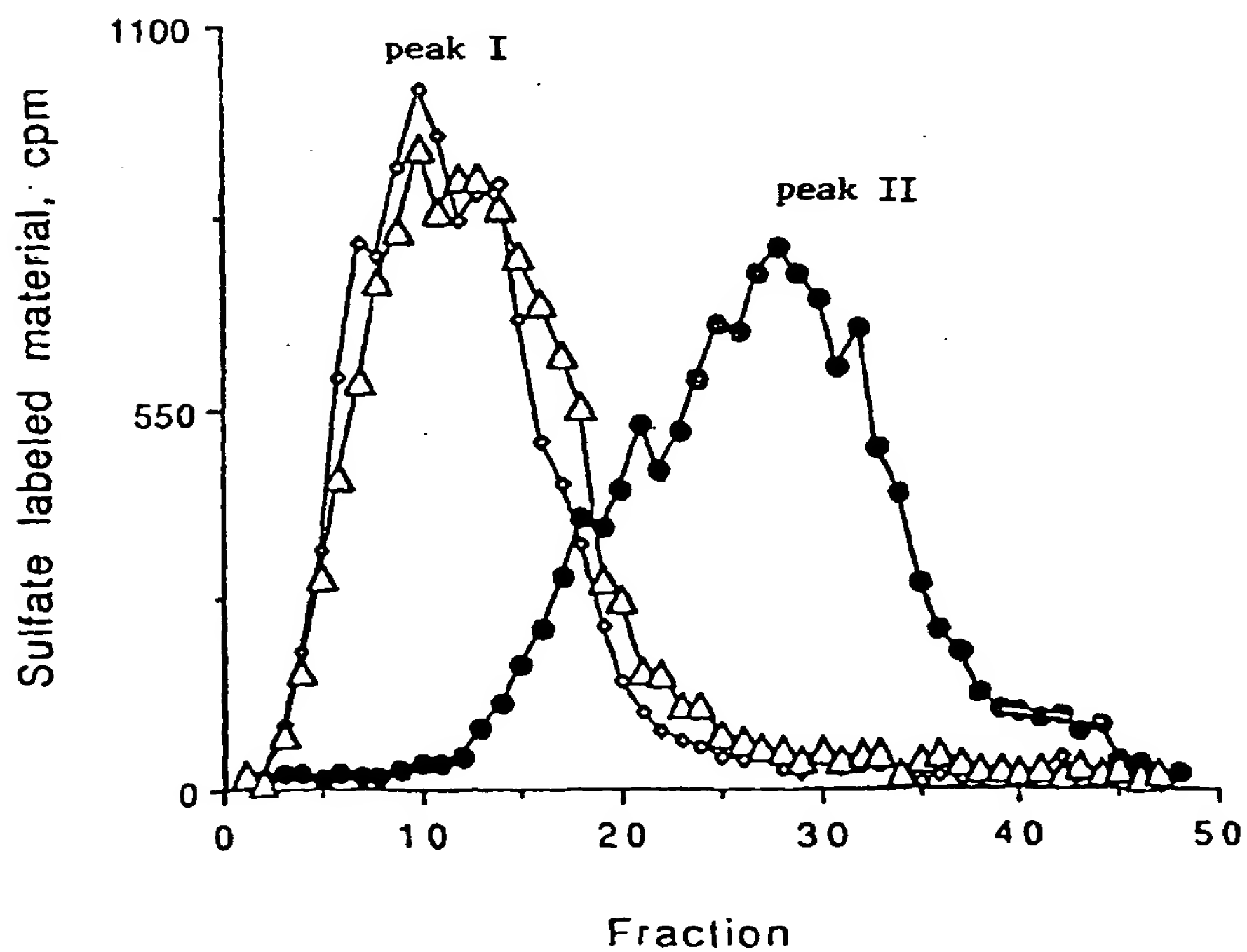


FIG. 6A

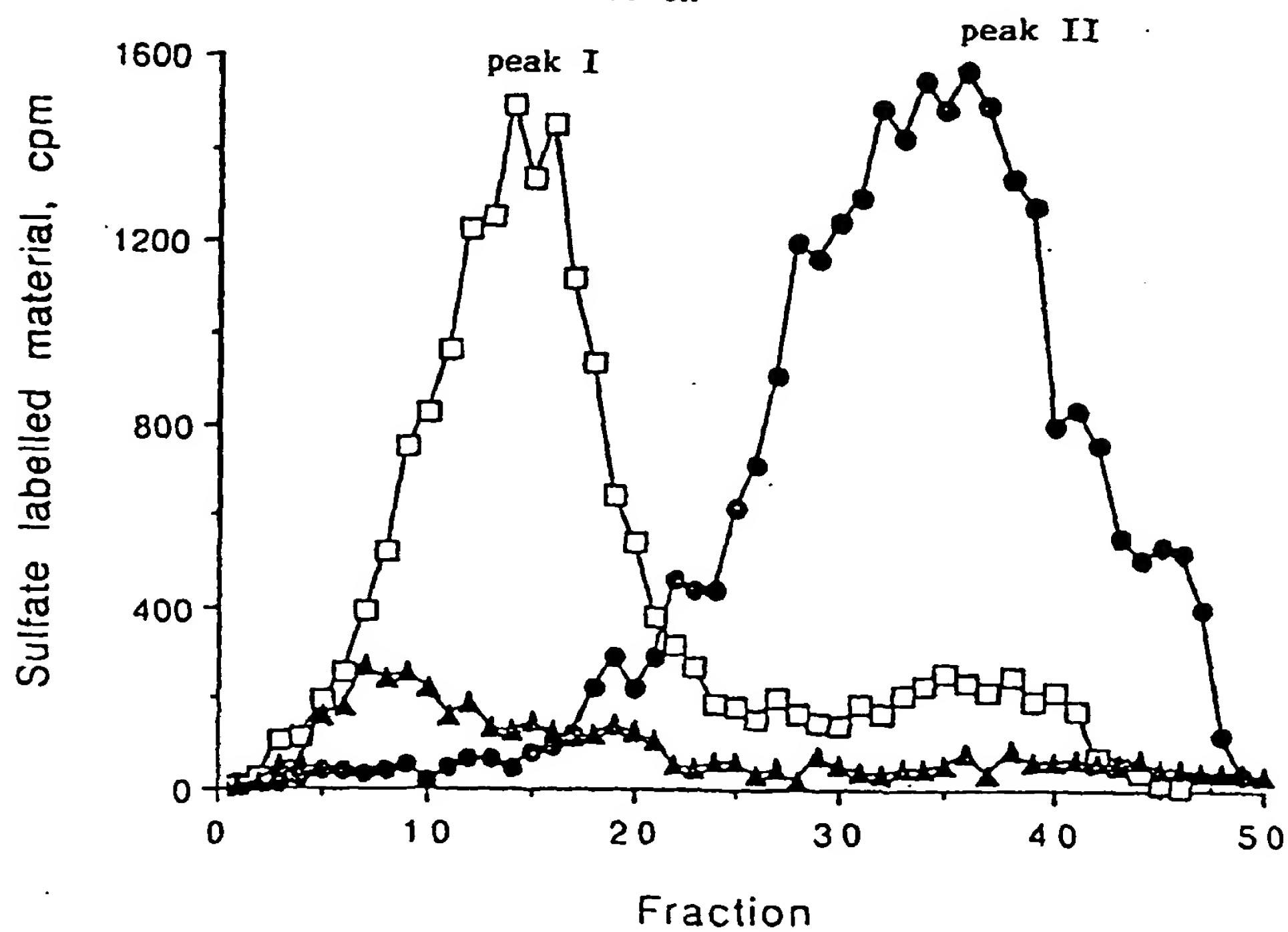


FIG. 6B

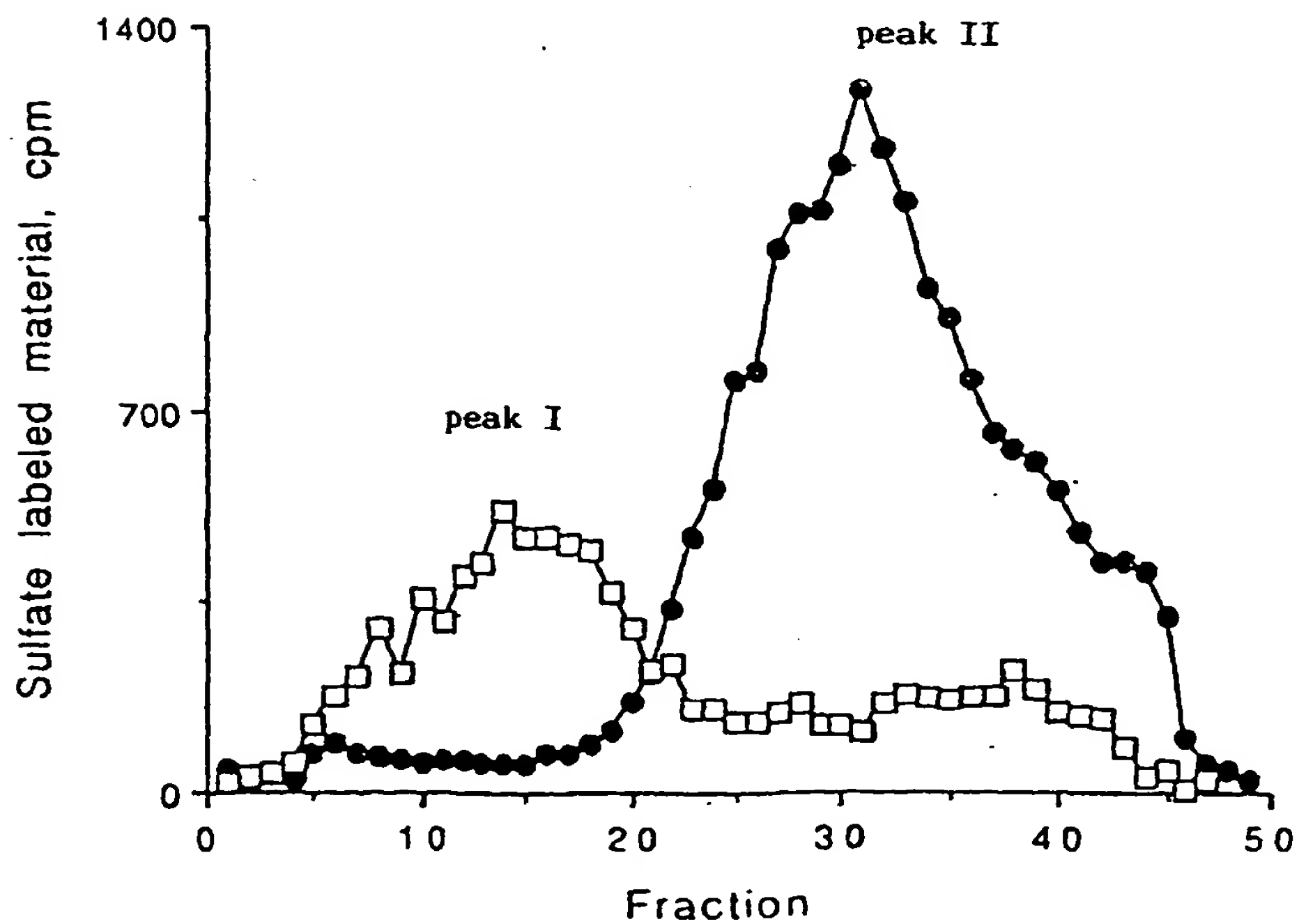


FIG. 7A

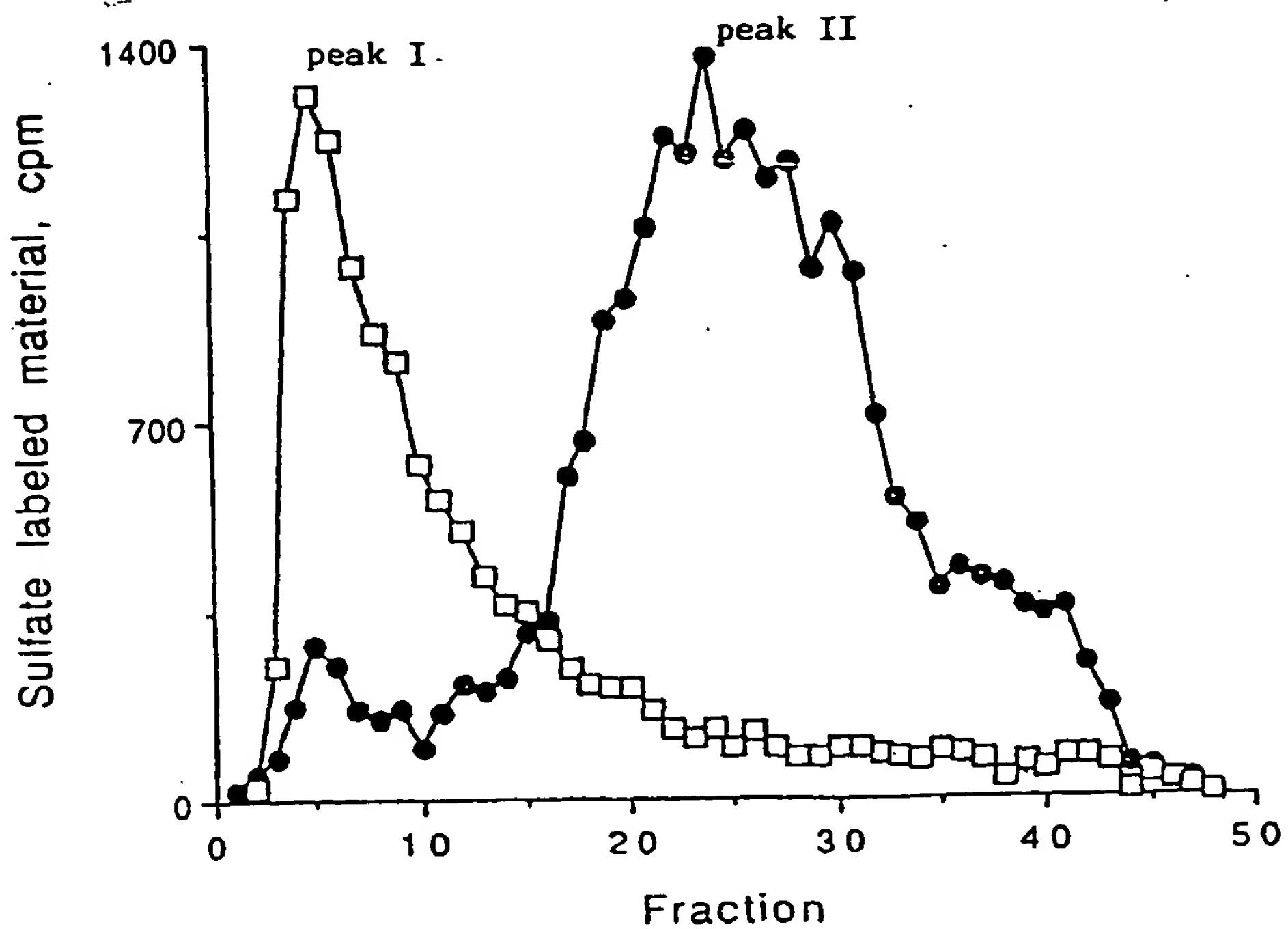


FIG. 7B

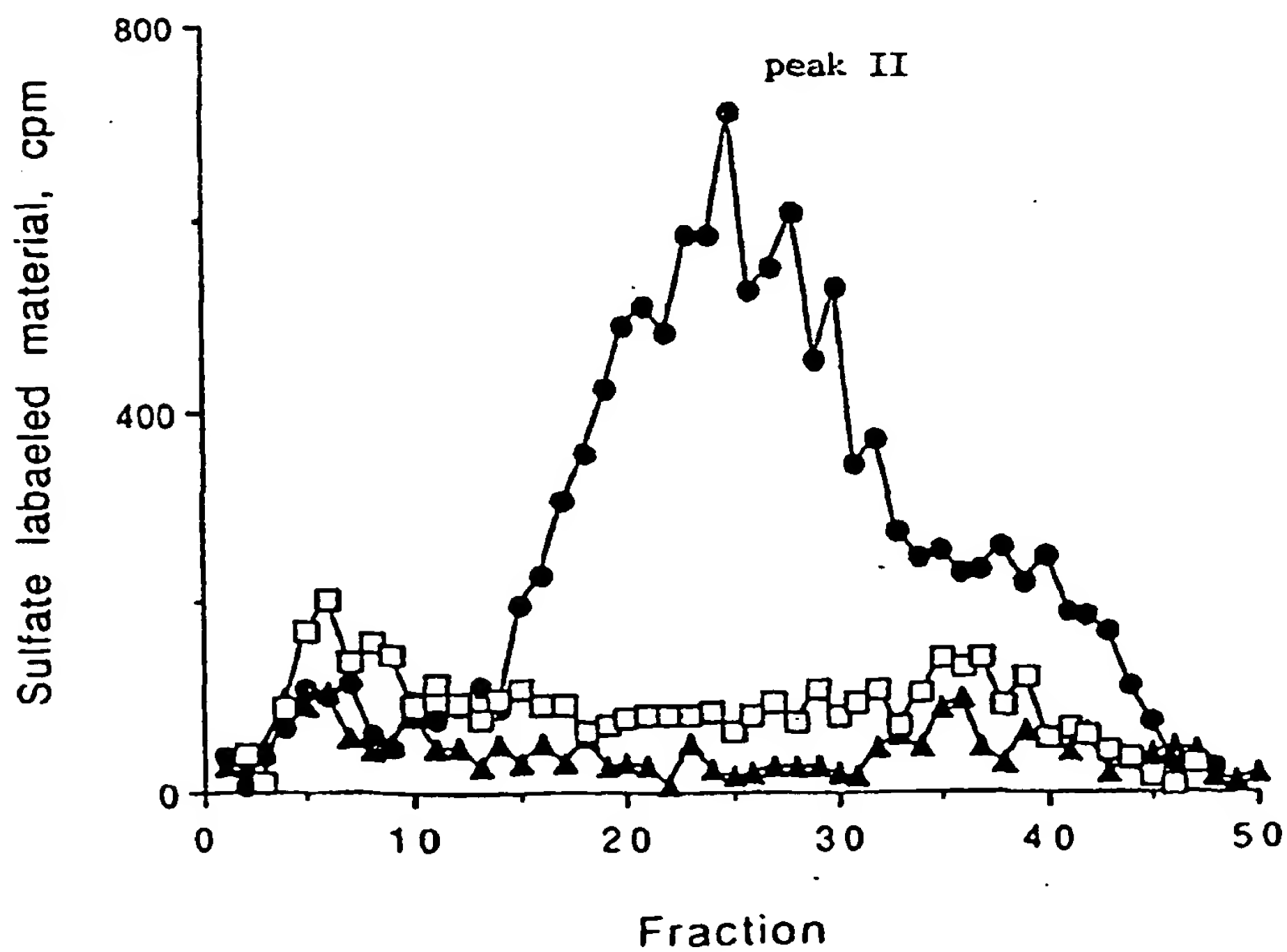




FIG. 8A

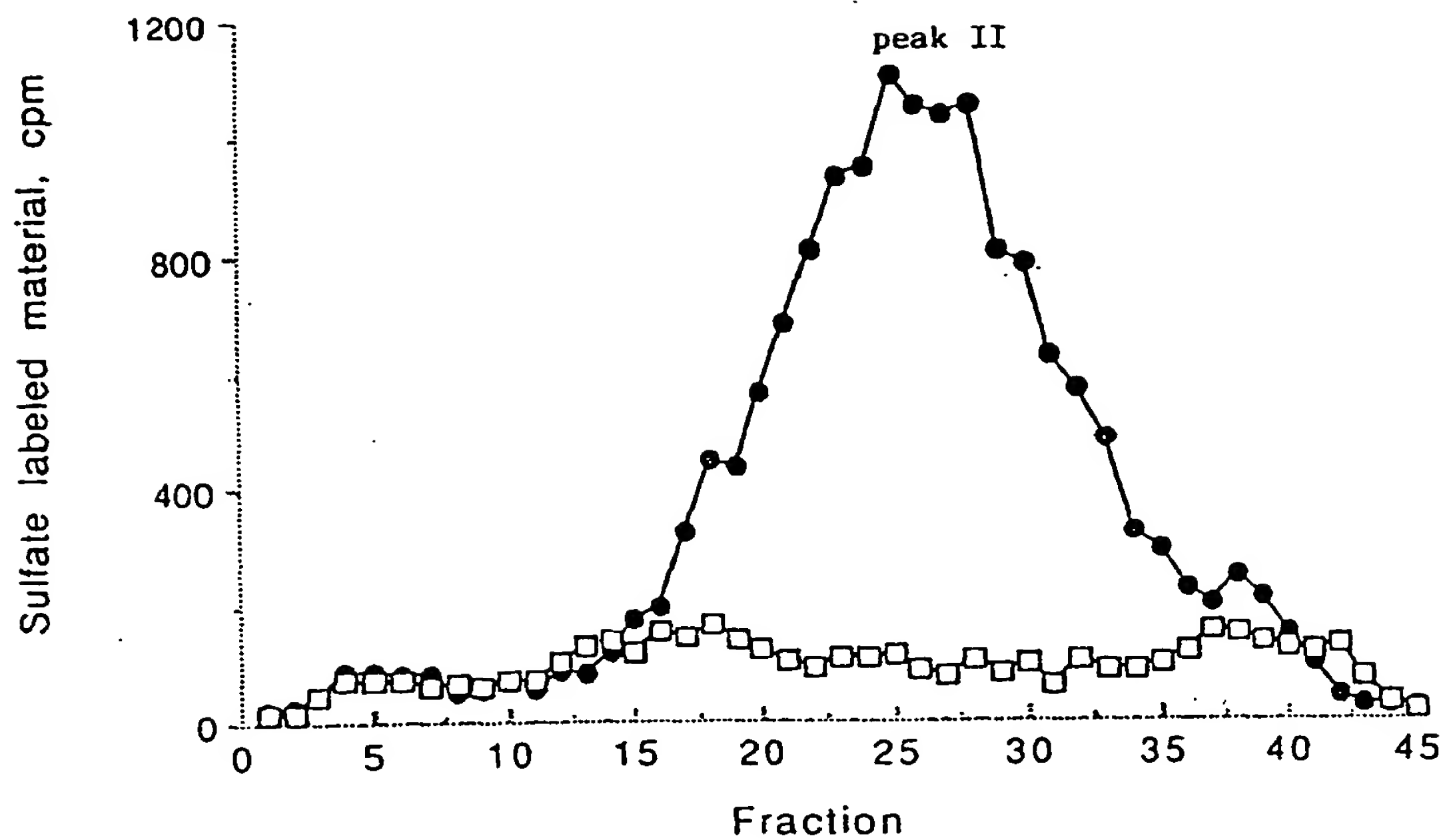


FIG. 8B

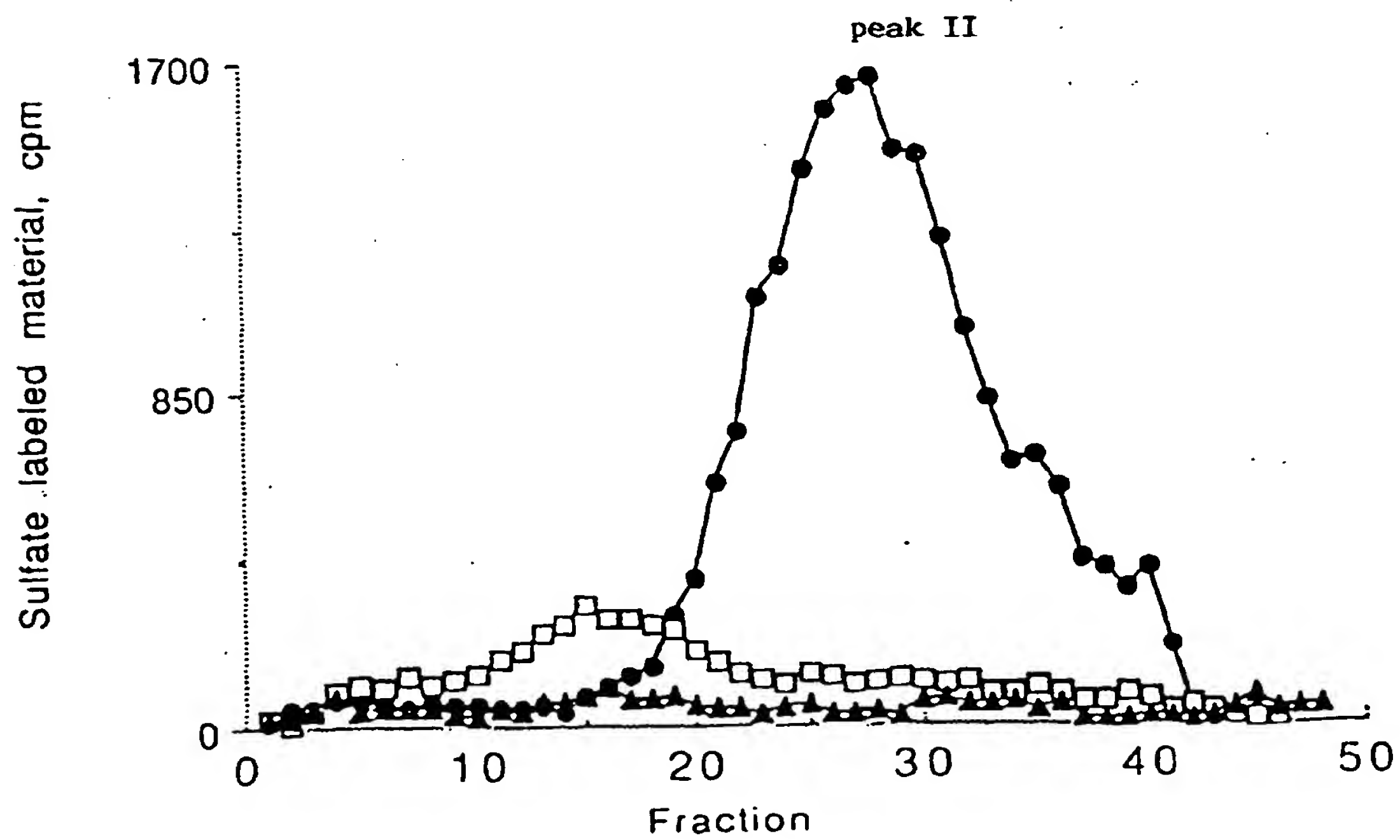


FIG. 9A

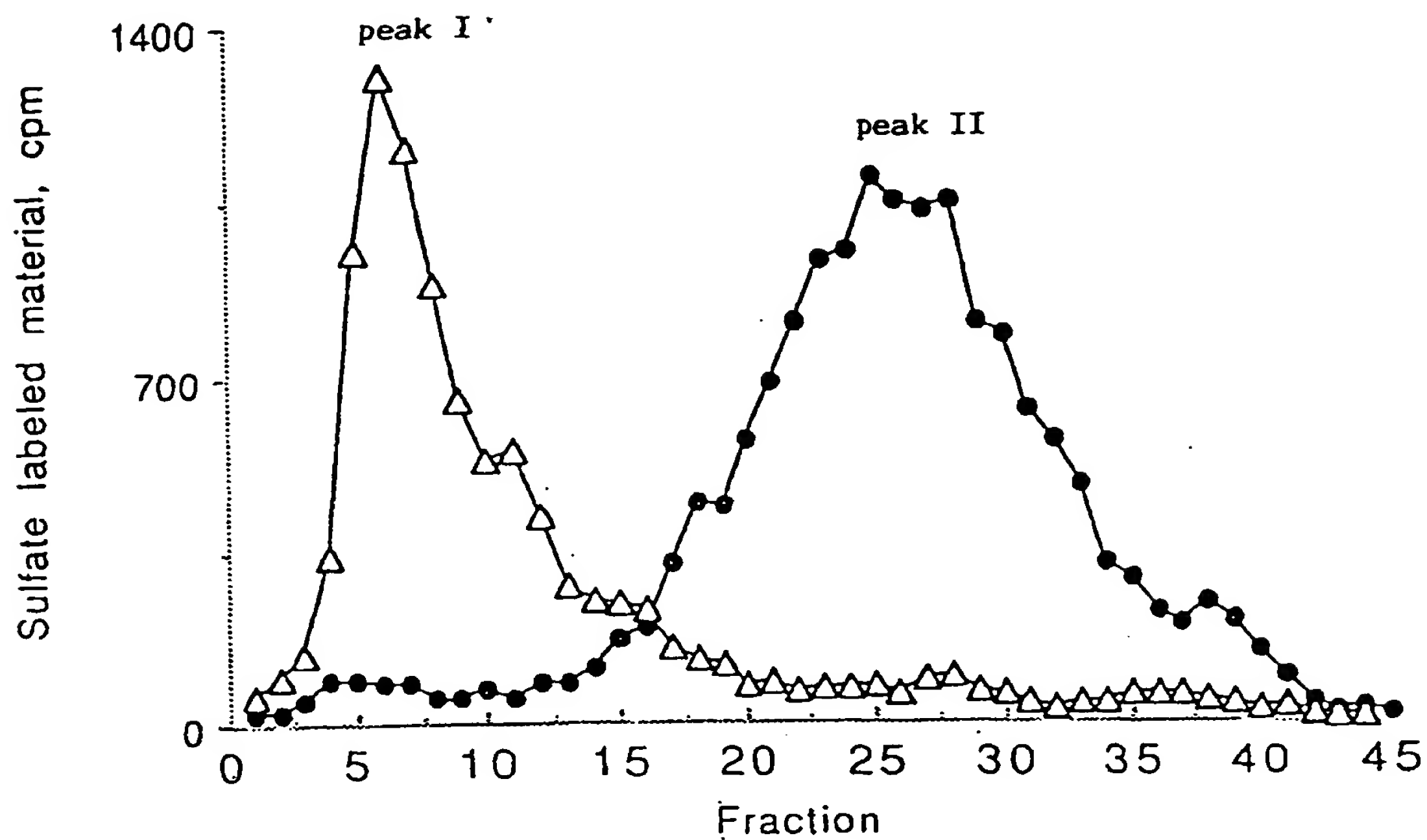


FIG. 9B

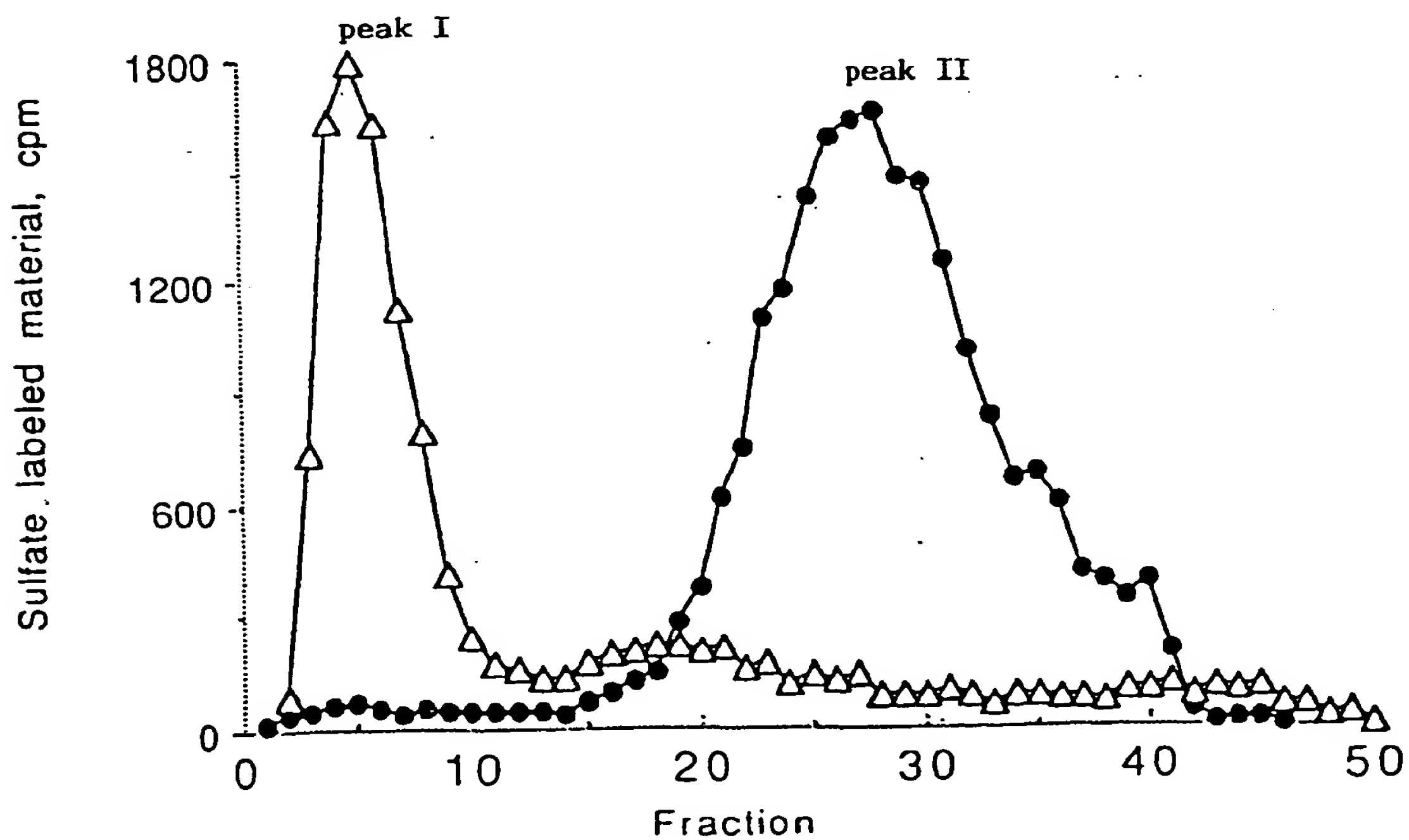


FIG. 10A

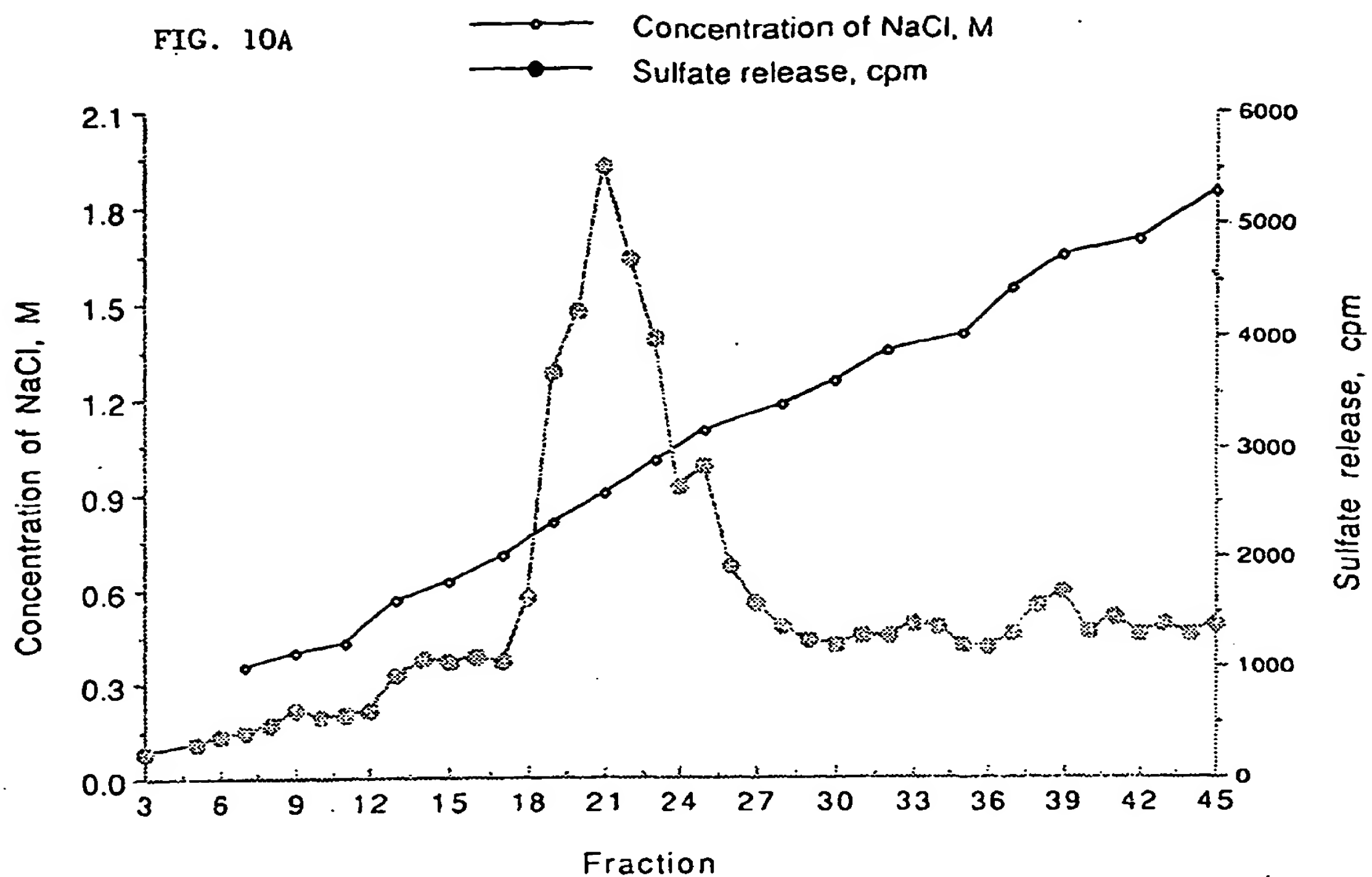


FIG. 10B

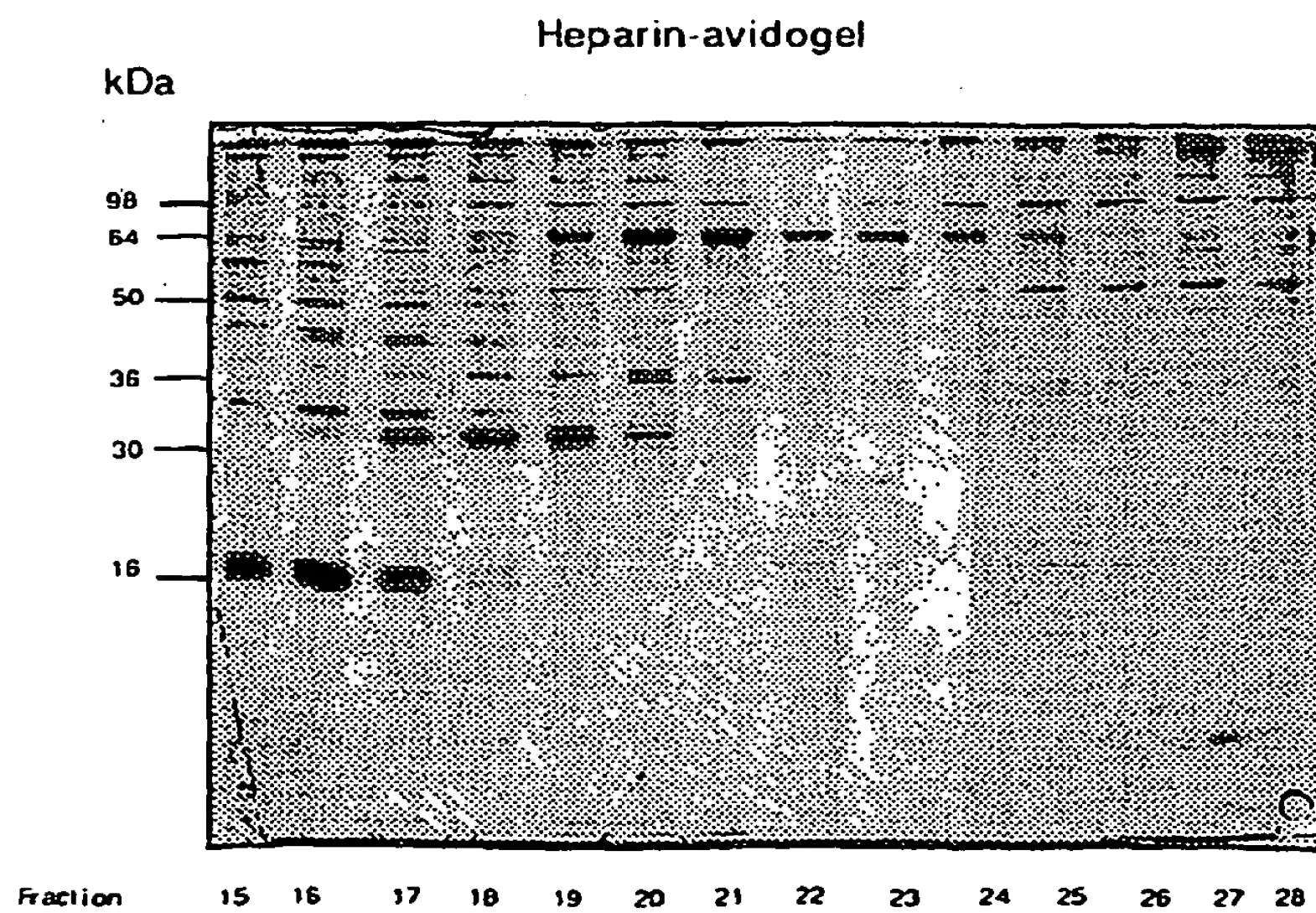


FIG. 11A

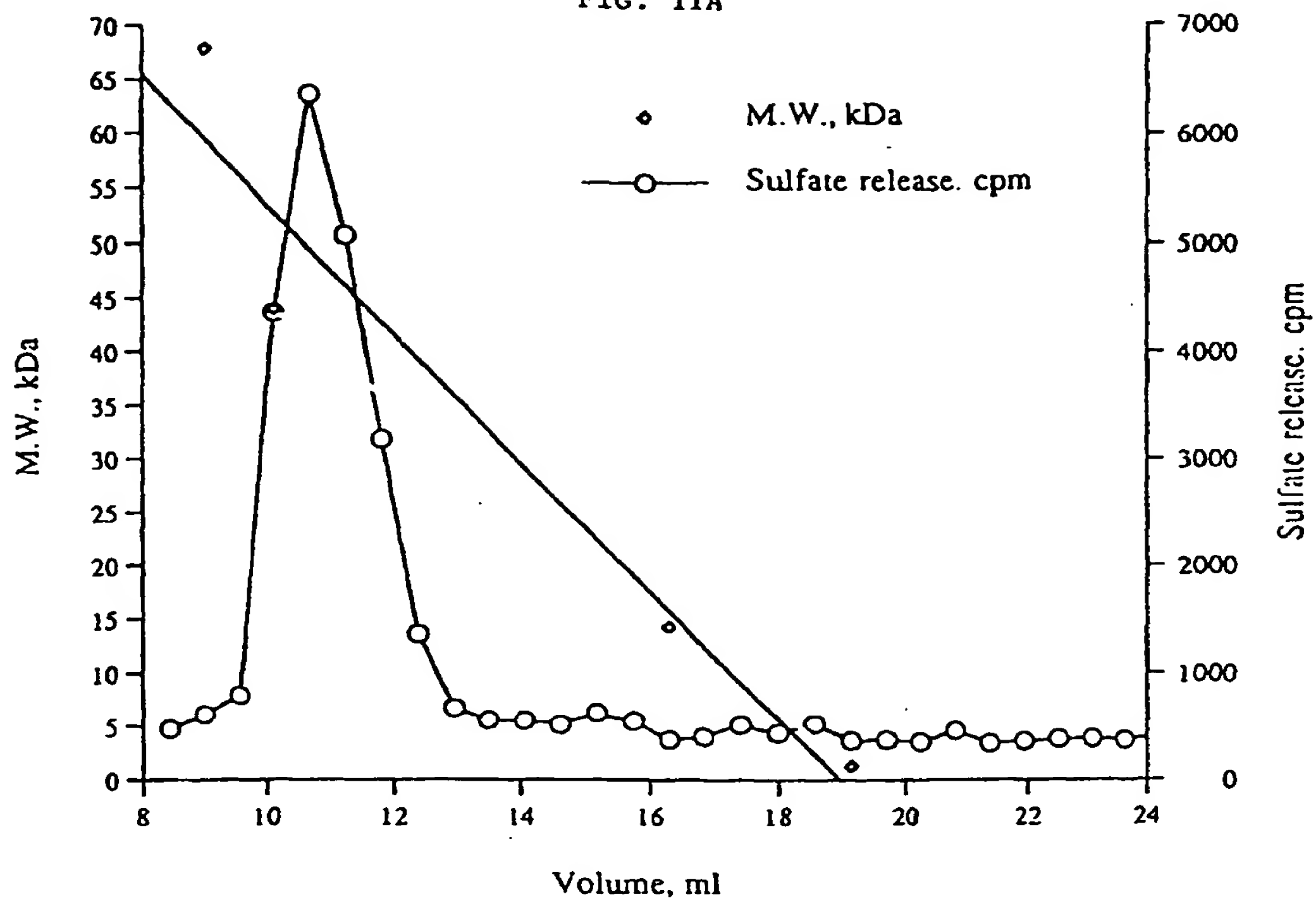
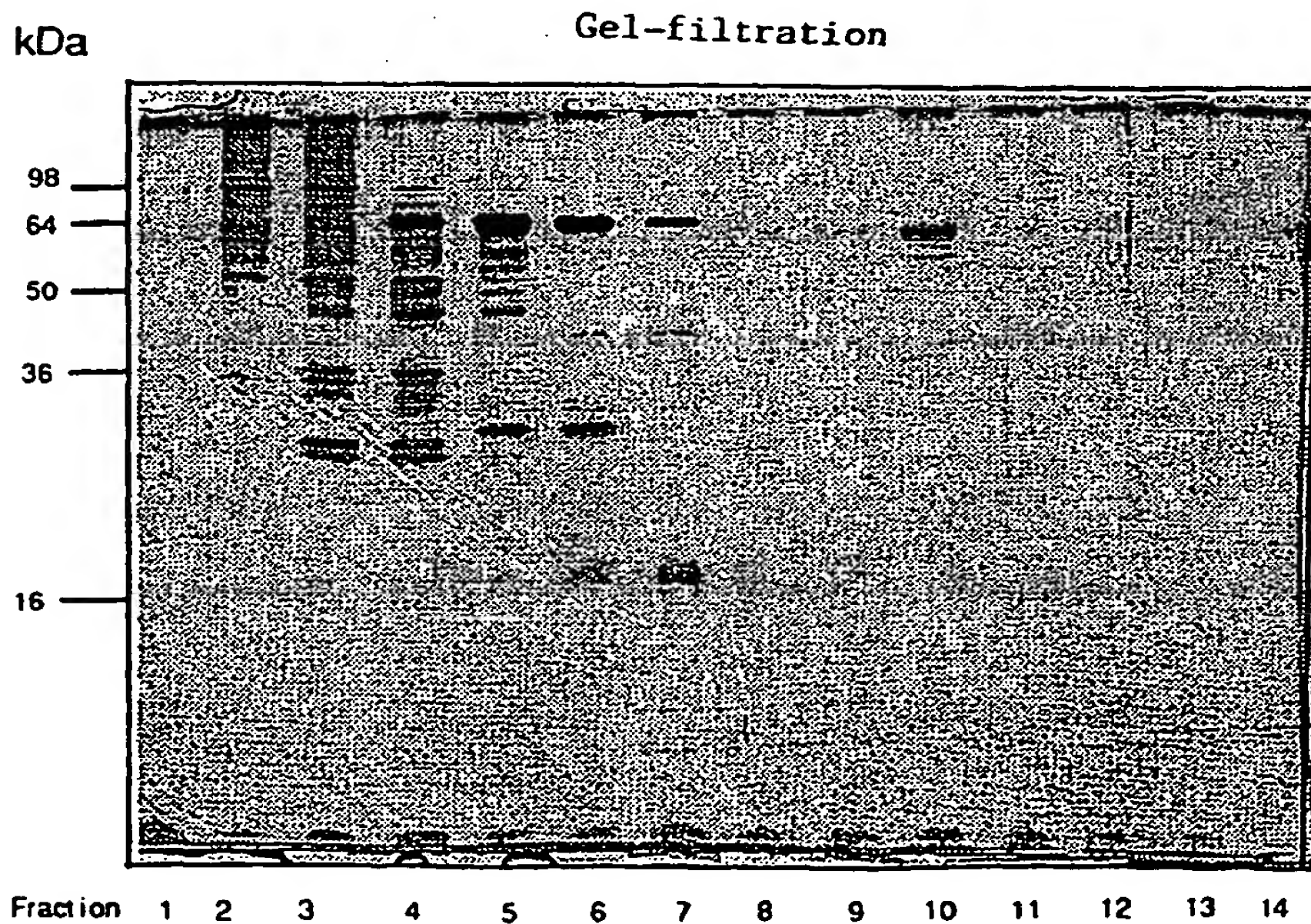


FIG. 11B



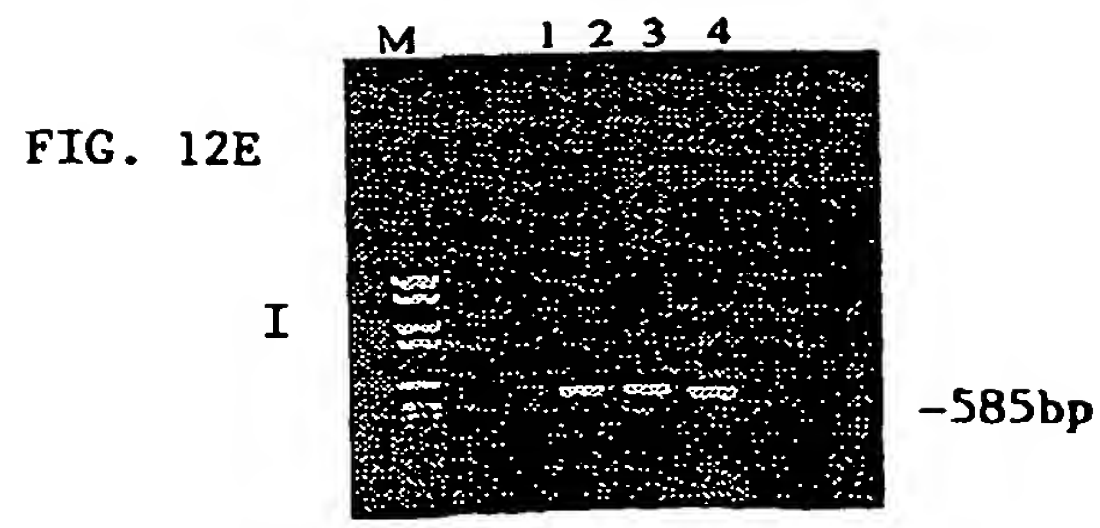
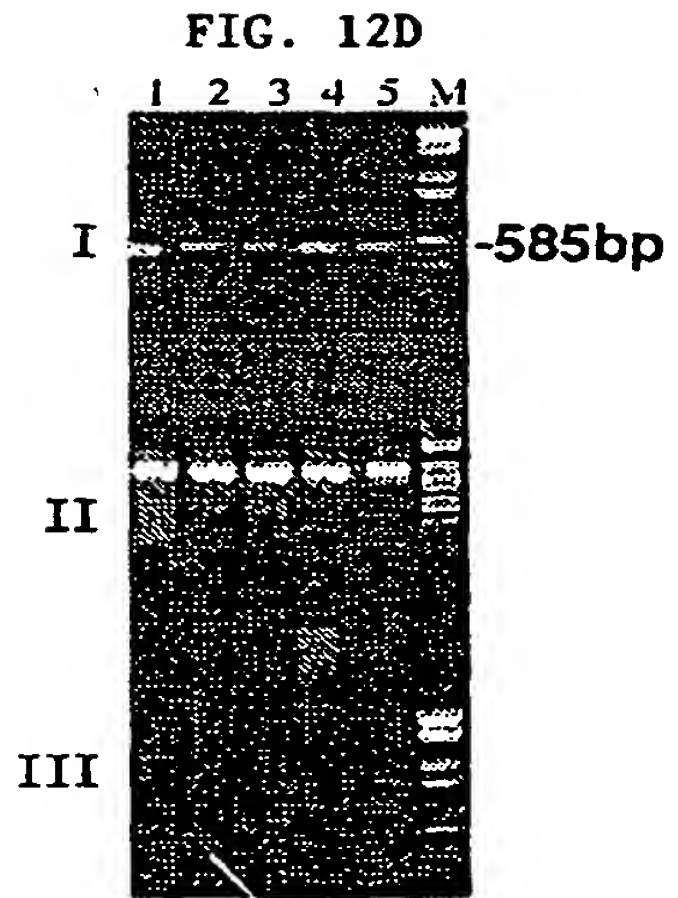
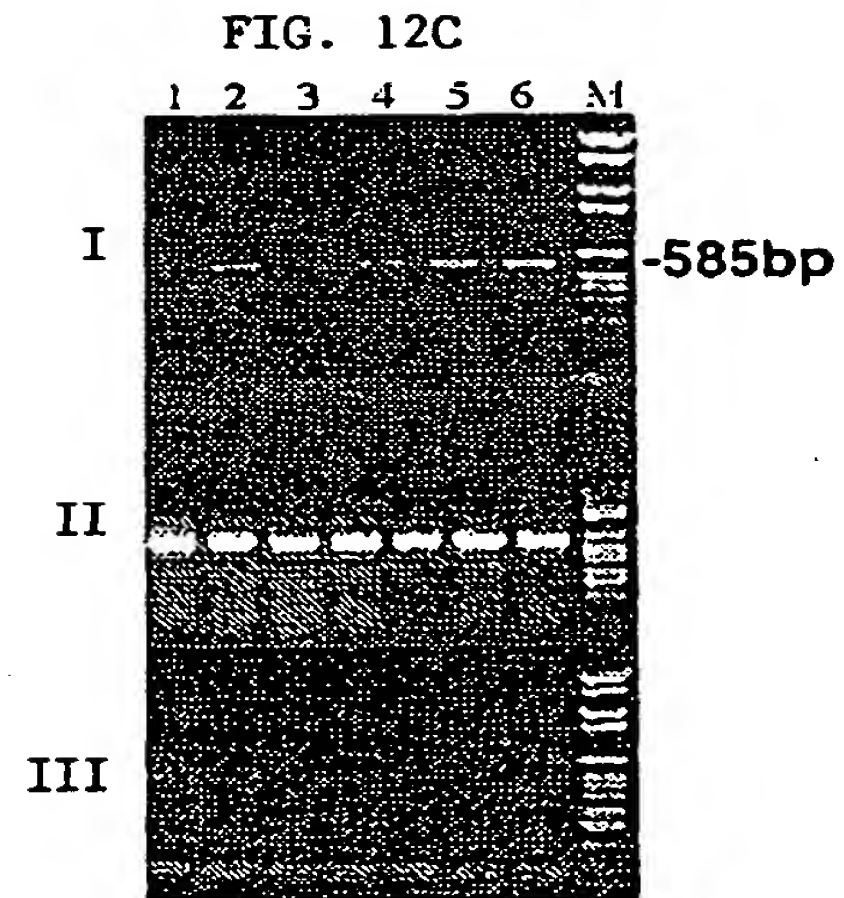
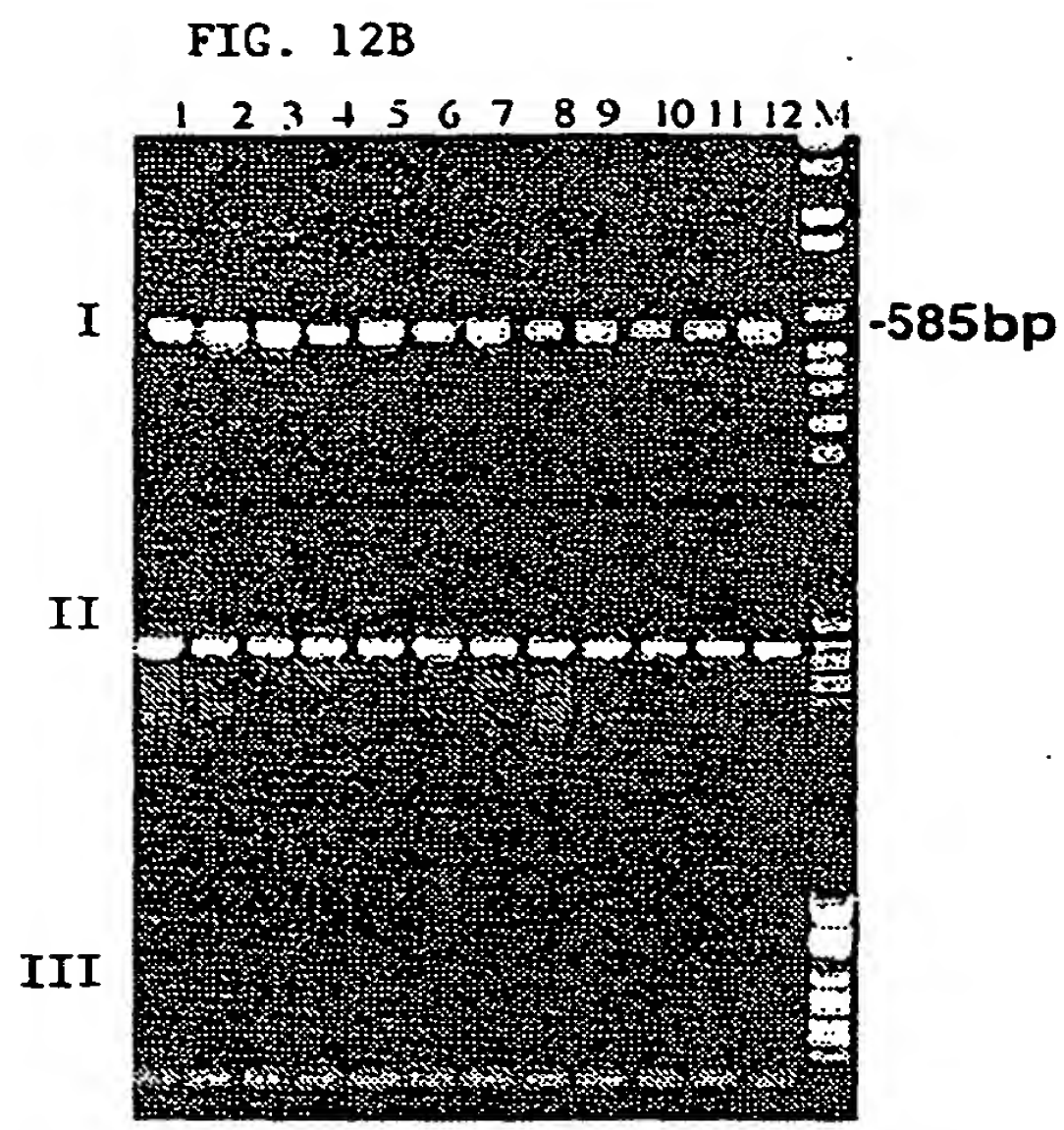
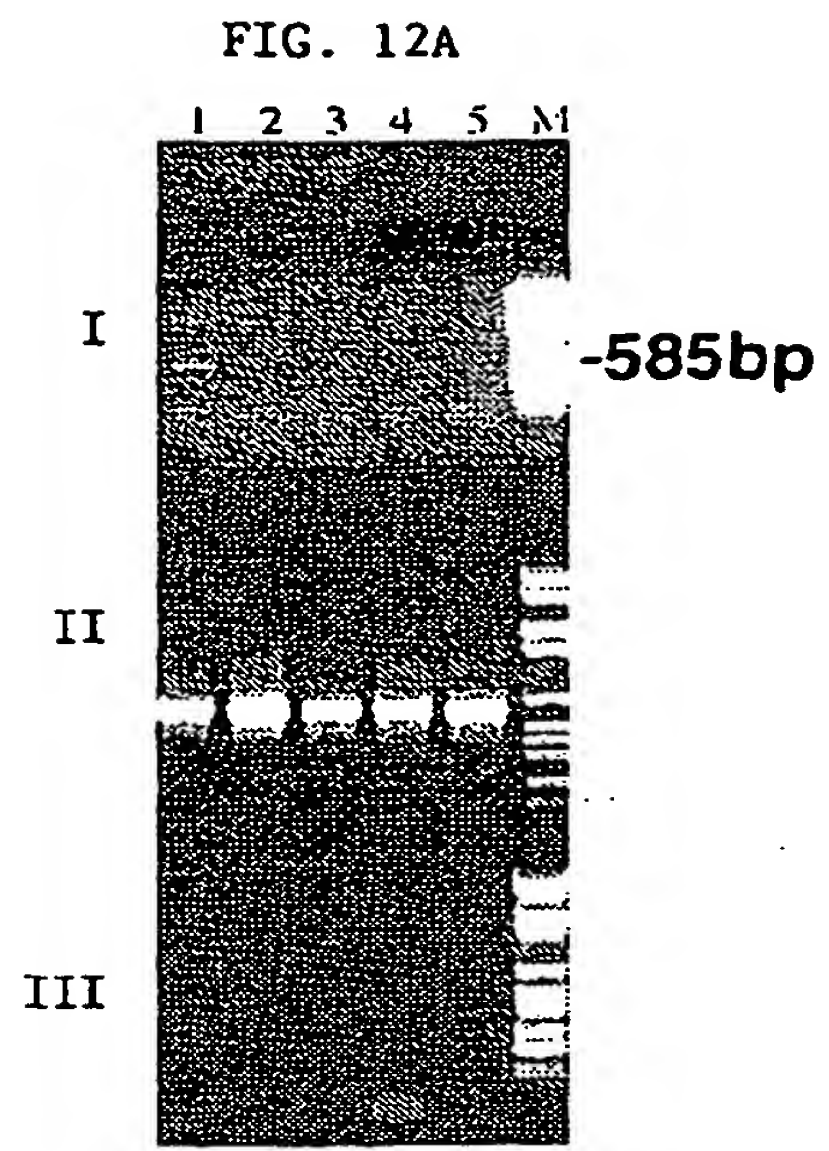




Fig. 13

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mouse CTGGCAAGAAGGTCTGGTTGGGAGAGACGAGCTCAGCTTACGGTGGCGGT 50
      |||||
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mouse GCACCCTTGCTGTCCAACACCTTTGCAGCTGGCTTTATGTGGCTGGATAA 100
      ||
human GCGCCCTTGCTATCCGACACCTTTGCAGCTGGCTTTATGTGGCTGGATAA 1165

mouse ATTGGGCCTGTCAGCCCAGATGGGCATAGAAGTCGTGATGAGGCAGGTGT 150
      |||||
human ATTGGGCCTGTCAGCCCAGATGGGAATAGAAGTGGTGTGATGAGGCAAGTAT 1215

mouse TCTTCGGAGCAGGCAACTACCACTTAGTGGATGAAACTTTGAGCCTTTA 200
      |||||
human TCTTTGGAGCAGGAAACTACCATTTAGTGGATGAAACTTCGATCCTTTA 1265

mouse CCTGATTACTGGCTCTCTCTTCTGTTCAAGAACTGGTAGGTCCCAGGGT 250
      |||||
human CCTGATTATTGGCTATCTCTTCTGTTCAAGAAATTGGTGGGCACCAAGGT 1315

mouse GTTACTGTCAAGAGTGAAAGGCCAGACAGGAGCAAACCTCCGAGTGTATC 300
      |||||
human GTTAATGGCAAGCGTGCAAGGTTCAAAGAGAAGGAAGCTTCGAGTATACC 1365

mouse TCCACTGCACTAACGTCTATCACCCACGATATCAGGAAGGAGATCTAACT 350
      |||||
human TTCATTGCACAAACACTGACAATCCAAGGTATAAAGAAGGAGATTTAACT 1415

mouse CTGTATGTCCTGAACCTCCATAATGTCACCAAGCACTTGAAGGTACCGCC 400
      |||||
human CTGTATGCCATAAACCTCCATAACGTCACCAAGTACTTGCGGTTACCCTA 1465

mouse TCCETTGTTCAGGAAACCAGTGGATACGTACCTTCTGAAGCCTTCGGGGC 450
      |||||
human TCCTTTTCTAACAAGCAAGTGGATAAATACCTTCTAAGACCTTTGGGAC 1515

mouse CGGATGGATTACTTTCCAAATCTGTCCAACCTGAACGGTCAAATTCTGAAG 500
      |||||
human CTCATGGATTACTTTCCAAATCTGTCCAACCTCAATGGTCTAACTCTAAAG 1565

mouse ATGGTGGATGAGCAGACCCTGCCAGCTTTGACAGAAAAACCTCTCCCCGC 550
      |||||
human ATGGTGGATGATCAAACCTTGCCACCTTTAATGGAAAAACCTCTCCGGCC 1615

mouse AGGAAGTGCACTAAGCCTGCCTGCCTTTTCTATGGTTTTTTTGTGATAA 600
      |||||
human AGGAAGTTCAGTGGGCTTGCCAGCTTTCTCATATAGTTTTTTTGTGATAA 1665

mouse GAAATGCCAAAATCGCTGCTTGTATATGAAAATAAAA 637
      |||||
human GAAATGCCAAAGTTGCTGCTTGCATCTGAAAATAAAA 1702

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FIG. 14

